ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1973

PART V

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1972 through June 30, 1973

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Annual Report of the Director

Division of Cancer Treatment

C. G. Zubrod, M.D.

The annual report for FY 1973 finds the Division of Cancer Treatment in the midst of program changes imposed in large part by the emergence of research opportunities and to some extent by the assumption of new responsibilities. Formerly, the objectives of the program were to develop drugs for the treatment of cancer patients and with these drugs to fashion a theoretical base for the chemotherapy of cancer. The successful achievement of major portions of these objectives for certain uncommon tumors has defined new research avenues to be followed for control of the more common tumors and this evolution is responsible for most of the program changes. In addition, the Division has recently been charged with the responsibility for research and development for all modalities of cancer treatment. In the execution of these new responsibilities, program modifications will be complementary to the evolutionary changes.

At the present time in the drug development portion of the program, there exist well-studied systems for predicting the efficacy, safety and pharmacologic disposition of active anti-tumor drugs for patients. For certain types of cancer these drugs when used alone, or in conjunction with surgery or X-irradiation, will bring about normal life expectancy in a majority of patients adequately treated. Usually, several drugs used in combination give far better results than a single drug. These drug-sensitive cancers are rapidly growing, generally not well localized in a single lump, have a high percentage of their cells synthesizing DNA at any one point in time, and are "young" tumors, that is, they are discovered early in their natural history. Similar drug treatment in the more common, slowly growing tumors, has not resulted in normal life expectancy, although drug administration often achieves temporary disappearance of the tumor. These tumors are "old", in the sense that they are discovered late in their natural history. A good deal is known about the biologic differences between "young" and "old" tumors. While control of 100% of young tumors has not been achieved, more than half of the job is finished, and the road to added improvements is clearly defined. It is also evident that further reduction in mortality from old tumors cannot be achieved by any single modality of treatment. Both in experimental systems and in preliminary studies in patients, there is a modest probability that reduction in mortality due to old tumors can be obtained by combining treatment modalities. The objective of the present program is to achieve reductions in mortality of the common, old tumors, through studies of combination treatment modalities.

While the responsibility of the Division for combined modality research can be precisely stated, the question of research and development for improving single modality treatment must be faced. It is the tentative decision of the National Cancer Institute that research in surgery, radiation therapy and immunotherapy as single modalities is best supported through existing mechanisms, and it is not intended at this time to use the Division of Cancer Treatment to undertake new programs in these areas. The NCI will continue to monitor the support of these programs.

Responsibility for research in chemotherapy, as a single modality, will continue to be in the Division, but major changes have been made in its emphases. Before a scientific basis for cancer chemotherapy existed, it was necessary to shape the experimental base from clinical leads. These clinical leads were largely among the young, rapidly growing tumors. As more drugs were developed some were found to have significant but temporary effects upon the old tumors. For the last five years the staff has been exploiting these drugs active against old tumors, to define and calibrate a new screen that would be useful in discovering drugs for both young and old tumors. Such a screen has been developed and is now in operation.

This broader search requires a larger input of chemicals to the screen and the use of more animals. In order to keep the cost at a reasonable level, studies have been made of a "mini screen", which would make it possible to increase the number of chemicals examined without corresponding increase in cost. Field tests of the mini screen are complete, and it will replace the current screen.

The aiming of the drug development apparatus in the direction of the old tumors is being achieved by another means. There is an increasing understanding of the correlations between chemical structure of anti-tumor drugs and their selective activity against cancer cells. To an increasing extent, the synthesis of new chemicals for screening is being dictated by structure-activity considerations. The recent recognition of adriamycin and other anthracycline analogues as anti-tumor agents with activity in a broad spectrum of old tumors presents a new opportunity for understanding the relation of structural features to capacity to control old tumors, and for the synthesis and trial of those molecular configurations most likely to result in improved selective toxicity for the old tumors.

A drug development program must screen potential drugs for safety as well as for efficacy. The highly successful experimental systems that have been developed will, of course, be of equal use for the study of drugs in patients with either old or young tumors. The same conclusions cannot be reached for studies of pharmacologic disposition of drugs. The usefulness of pharmacologic examination of a drug is that from the data one can translate an effective and safe drug regimen in the animal to the optimal drug regimen for patients. The experience in the use of drugs for controlling young tumors has shown that optimal regimens can be designed only when one integrates cell kinetic data of the tumor and bone marrow and the pharmacokinetic data of the drug. Since the cell population kinetics of old tumors differ from those of young tumors, there is a need for different dose regimens and, hence, for continued alertness to match pharmacologic studies to the appropriate cell population kinetics.

While the drug development program has previously been built around models for the study of single agents, the impressive superiority of combinations of drugs in clinical cancer has emphasized the need for efficacy and safety screens for drug combinations. The variables are so numerous and the expense of a systematic examination is so great, that there is a serious question as to the feasibility and usefulness of models for drug combinations.

The Division continues to explore several approaches to such models, but cannot propose a final solution. Models for the study of combined modality therapy are less complex and are in use.

In summary, the Division has been changing its goals in keeping with new opportunities and responsibilities. As these changes come about, it is important to examine the balance between short range and long range goals. The most immediate goal is to use all of our present knowledge on therapy to reduce mortality and morbidity in as many types of cancer as possible. New knowledge about therapeutic control of both young and old tumors is developing rapidly. The Division will expend most of the new funds for the next fiscal year in the support of all those opportunities ready for clinical exploration and application. Because these opportunities exist in a number of different areas--in the Division's direct research as well as in contract and grant supported investigations--and because they involve a variety of institutions, task forces and study groups, especial attention will be paid to monitoring and coordination.

The long range goals are the development of better drugs, and newer concepts of combined modality therapy. There is a need for vigorous program in both areas, and this will be in part achieved by a more fundamental understanding of the empirical successes in controlling cancer by drugs. A good deal of support for these long range objectives already exists within the Division, as well as in grant supported studies. Again, there is a need and a determination to be aware of new findings, and to use the new knowledge to devise better therapy.

CODE FOR DIVISION OF CANCER TREATMENT PROGRAM LOGIC

Stage	Code	Description
I	1XX	Acquisition of new agents (includes studies of cellular control mechanisms).
IIA	2AX	Determination of anti-tumor activity of new agents.
IIB	2B1	Develop acceptable experimental formulation.
IIB	2B2	Detailed evaluation of new agents--dose, route, and schedule dependency (in vivo and in vitro systems). Cell kinetics.
IIB	2B3	Initial animal pharmacology studies; evaluation for absorption and anti-tumor activity after oral administration.
IIB	2B4	Procurement of sufficient amounts of new agent for pre-clinical studies.
III	3X1	Toxicology and pharmacology in large animals (dogs and monkeys). Pharmacokinetics.
III	3X2	Production and formulation for clinical trials.
IV	4X1	Clinical Trials*-Phase I. Determination of tolerated dose. Cell kinetics.
IV	4X2	Clinical pharmacology--initial pharmacologic studies in man associated with early clinical trials. Pharmacokinetics.
V	5XX	Clinical Trials*--Phase II. Initial therapeutic trials. Development of "markers".
VI	6X1	Clinical Trials*--Phase III. Initial eradicative trials in man. Agent used singly or in combination with other agents in comparative study. Research, development and subsequent evaluation in supportive care (protected environment, platelet and leukocyte transfusions, antibiotics, etc.).
VI	6X2	Toxicologic and pharmacologic studies in large animals and man associated with further development of a drug. Microsomal enzyme. Molecular biology.
VII	7XX	Clinical Trials*--adjuvant studies (radiotherapy, surgery, immunotherapy). Determination of role of agent in total care. Rehabilitation.
---	8XX	Unassigned. Includes administration, dissemination of information to medical and scientific community. Generalized D.P.

* Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TYPE OF ACTIVITY BY LINEAR ARRAY CLASSIFICATION		NO. OF CONTRACTS	ANNUAL LEVEL[1]		(PERCENT)
	TOTAL	200[2]	39,349,635		
STAGE I			8,158,351		(20.7)
1XX	Acquisition of New Agents	107	8,158,351	(20.7)	
STAGE IIA			10,169,272		(25.8)
2AX	Determination of Anti-tumor Activity	87	10,169,272	(25.8)	
STAGE IIB			6,490,205		(16.5)
2B1	Experimental Formulation Development	5	166,213	(00.4)	
2B2	Detailed Evaluation of New Agents	79	5,325,965	(13.5)	
2B3	Initial Annual Pharm. Studies	0	0	(00.0)	
2B4	Procure. of Agents for Preclinical Studies	8	998,027	(02.6)	
STAGE III			5,293,117		(13.5)
3X1	Toxicology and Pharm. in Large Animals	27	2,919,794	(07.4)	
3X2	Product. and Formulation for Clinical Trials	20	2,373,323	(06.1)	
STAGE IV			730,930		(01.9)
4X1	Clinical Trials-Phase I	5	135,728	(00.4)	
4X2	Initial Clinical Pharm. Studies in Man	12	595,202	(01.5)	
STAGE V			1,573,168		(04.0)
5XX	Clinical Trials-Phase II	30	1,573,168	(04.0)	
STAGE VI			5.509,186		(14.0)
6X1	Clinical Trials-Phase III	32	2,467,273	(06.3)	
6X2	Tox. & Pharm. Studies in Large Animals and Man	29	3,041,913	(07.7)	
STAGE VII			1,214,783		(03.1)
7XX	Clinical Trials-Adjuvant Studies	15	1,214,783	(03.1)	
UNASSIGNED			210,623		(00.5)
8XX	Administration and Information Functions	9	210,623	(00.5)	

1/ Funds obligated during Fiscal Year 1973. Not included are funds utilized for direct purchase of clinical drugs (approximately $3,553,000), reimbursable agreements with the Veterans Administration ($1,060,000), reimbursable agreement with the Public Health Service in Baltimore ($2,455,000), and about eleven new contracts not negotiated as of this report ($770,465).

2/ Since many contracts involve more than one activity, the totals of Stages I through VII and the unassigned totals category do not correspond with the total number of contracts.

TABLE II

ANALYSIS OF CONTRACTS

BY ACTIVITY

VITY

CONTRACT	DOLLAR LEVEL
GE I: ACQUISITION OF NEW AGENTS	
AGRICULT.,DEPT.OF (64-1)	328,000
ARIZONA BOARD OF REGENTS ARIZONA STATE UNIV. (71-2308)	46,896
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (67-1484)	121,000
ARS/SPRAGUE-DAWLEY (71-2007)	92,372
ASH STEVENS,INC. (71-2314)	69,867
ASSOCIATED BIOMEDIC SYSTEMS, INC. (70-2271)	28,320
BATTELLE MEM.INST (70-2008)	10,372
BATTELLE MEM.INST. (69-2251)	25,900
BATTELLE MEM.INST. (71-2182)	66,030
BELLAIRE ACRES (72-729)	0
BELLAIRE ACRES (73-805)	7,718
BELLAIRE ACRES (73-832)	27,520
BRISTOL-MYERS CO. (69-35)	682,309
BRITISH COLUMBIA, UNIV. OF (72-3223)	79,780
CENTRAL DRUG RES. INST.(P.L.480)	0
CHAS. RIV. BREEDING LABS.(73-839)	30,679
CHAS. RIV. BREEDING LABS.(73-807)	8,810
CHAS.RIV.BREEDING LABS.(72-730)	0
CHAS.RIV.BREEDING LABS.(72-2004)	104,645

TABLE II
(continued)

CONTRACT	DOLLAR LEV
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
CHEM.APSTR.SERVICE (65-1021)	129,40
COLLABORATIVE RES., INC. (71-2326)	49,34
COLLABORATIVE RES., INC. (66-506)	16,91
DELAWARE, UNIV.OF (69-2115)	20,4
DOW CHEMICAL CO. (71-2313)	90,50
FLOW RES.ANIM.,INC. (73-838)	24,74
GEORGIA, UNIV. OF (71-2311)	36,57
HARLAN INDUSTRIES (72-3218)	10,22
HARLAN INDUSTRIES (72-724)	
HARLAN INDUSTRIES (73-844)	8,51
HARLAN INDUSTRIES (73-845)	29,30
HAWAII, UNIV.OF (67-748)	55,00
HAZLETON LABS.,INC. (72-3704)	169,05
HORTON LABS. (72-361)	13
HORTON LABS. (73-837)	18,92
IIT RESEARCH INST. (68-1021)	76,70
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-2078)	85,99
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-3208)	50,37
INDIAN CANCER RES. CENTRE (P.L.480)	
INST.JULES BORDET (72-2053)	4,95

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (73-3720)	24,839
JACKSON LAB. (72-1171)	13,124
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	11,300
KANSAS, UNIV.OF (71-2010)	11,637
LAB. SUPPLY CO. (73-835)	8,300
LAB. SUPPLY CO. (73-840)	25,772
LAB.SUPPLY CO. (70-2041)	16,304
LAB.SUPPLY CO. (72-733)	0
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)	33,813
LITTLE,ARTHUR D.INC.(73-3727)	46,713
LITTLE,ARTHUR D.INC.(72-3257)	15,576
LITTON BIONETICS, INC.,(71-2340)	406,552
LITTON BIONETICS, INC. (72-3294)	56,280
MASON RES. INST. (71-2259)	6,400
MIAMI, UNIV. OF (70-2225)	72,037
MICHIGAN, REGENTS OF THE UNIV. OF (69-2004)	50,000
MICHIGAN,STATE OF, DEPT. OF PUBLIC HEALTH (73-3702)	45,115
MICRO. ASSOC. INC. (68-1283)	46,530
MICRO. ASSOC.,INC. (73-3728)	523,126
MIDWEST RES. INST. (73-3743)	331,165

TABLE II
(continued)

CONTRACT	DOLLAR LEV
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
MIDWEST RES.INST. (65-94)	99,98
MURPHY FARMS (73-868)	8,03
N.Y. STATE DEPT.OF HEALTH (70-2035)	12,90
NATIONAL ACADEMY OF SCIENCES #6 (64-44)	2,45
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)	7,46
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (73-3719)	492,39
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2187)	50,00
PENNWALT CORP. (71-2309)	60,00
POMONA COLLEGE (73-3703)	38,94
RESEARCH TRIANGLE INST.(69-2019)	139,95
RHODE ISLAND, UNIV. OF (71-2312)	39,45
SCRIPPS CLINIC & RES. FD. (72-2042)	23,50
SHEEHAN INST.FOR RES. (72-3719)	52,24
SIMONSEN LABS (72-725)	40
SIMONSEN LABS. (72-3219)	21,32
SIMONSEN LABS. (72-409)	75
SIMONSEN LABS. (73-816)	31,08
SIMONSEN LABS. (71-2101)	23,98
SOUTHERN ANIM.FARMS (72-732)	
SOUTHERN ANIM.FARMS (72-2013)	7,69

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
SOUTHERN ANIM.FARMS (73-863)	28,028
SOUTHERN RES.INST. (73-3712)	561,000
SOUTHERN RES.INST. (66-29)	160,390
SOUTHERN RES.INST. (71-2098)	106,573
SOUTHERN RES.INST. (71-2021)	76,000
SPARTAN RES. ANIM. INC.(73-878)	7,162
SPARTAN RES. ANIM., INC.(73-858)	7,158
STANFORD RES. INST. (73-3742)	238,896
STANFORD RES.INST (71-2070)	129,393
STARKS,C.P.,INC. (72-3705)	135,509
TACONIC FARMS (73-912)	19,110
TEXAS INBRED MICE (68-88)	48,880
UPJOHN COMPANY (73-3707)	391,610
UPJOHN COMPANY (72-3709)	35,978
UPJOHN COMPANY (68-1023)	25,595
UTAH, UNIV. OF (72-3710)	60,000
VALUE ENGINEERING COMPANY (73-3706)	79,604
VIRGINIA, UNIV.OF (71-2099)	129,000
VITRO LABORATORIES (69-58)	1,037
WARF INST., INC. (66-494)	306,890

TABLE II
(continued)

CONTRACT	DOLLAR LE
STAGE I: ACQUISITION OF NEW AGENTS (CONTINUED)	
WARF INST.,INC. (67-747)	
WASH.STATE UNIV. (72-2059)	18,9
WEIZMANN INST.OF SCIENCE(P.L.480)	
WISCONSIN,BOARD OF REGENTS OF THE UNIVERSITY OF (73-3733)	30,6
WISCONSIN,UNIV.OF (71-2275)	21,3
YALE UNIVERSITY (71-2339)	60,0
TOTAL	8,143,3

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

IVITY

CONTRACT	DOLLAR LEVEL
GE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY	
ARS/SPRAGUE-DAWLEY (70-2044)	35,691
ARS/SPRAGUE-DAWLEY (71-2342)	28,104
ARS/SPRAGUE-DAWLEY (71-2007)	405,010
BATTELLE MEM.INST (70-2008)	46,670
BATTELLE MEM.INST. (69-2251)	116,550
BATTELLE MEM.INST. (71-2182)	297,133
BELLAIRE ACRES (72-729)	0
BELLAIRE ACRES (73-805)	34,725
BELLAIRE ACRES (73-832)	123,832
BRISTOL-MYERS CO. (69-35)	103,139
CATHOLIC MED.CTR OF BROOKLYN & QUEENS, INC.(72-3703)	184,000
CENTRAL DRUG RES. INST.(P.L.480)	0
CHAS. RIV. BREEDING LABS.(73-839)	138,050
CHAS. RIV. BREEDING LABS.(73-807)	39,639
CHAS.RIV.BREEDING LABS.(72-730)	0
CHAS.RIV.BREEDING LABS.(71-2343)	22,500
CHAS.RIV.BREEDING LABS.(72-2004)	470,894
COLORADO,UNIV.OF (71-2186)	37,500
FLOW RES.ANIM.,INC. (73-838)	111,351

TABLE II
(continued)

CONTRACT	DOLLAR LEVI
TAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
HARLAN INDUSTRIES (72-3218)	65,700
HARLAN INDUSTRIES (72-724)	0
HARLAN INDUSTRIES (73-844)	38,329
HARLAN INDUSTRIES (73-845)	131,85
HAZLETON LABS.,INC. (72-3704)	382,200
HORTON LABS. (72-361)	598
HORTON LABS. (73-837)	85,176
IIT RESEARCH INST. (68-1021)	942,398
INDIAN CANCER RES. CENTRE (P.L.480)	0
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (73-3720)	99,353
JACKSON LAB. (72-1171)	87,484
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	14,125
KANSAS, UNIV.OF (71-2010)	52,364
LAB. SUPPLY CO. (73-835)	37,346
LAB. SUPPLY CO. (73-840)	115,970
LAB.SUPPLY CO. (70-2041)	73,362
LAB.SUPPLY CO. (72-733)	0
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)	152,15
LITTLE,ARTHUR D.INC.(73-3727)	653,951
LITTLE,ARTHUR D.INC.(72-3257)	80,270

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
LITTON BIONETICS, INC. (72-3294)	253,260
LITTON BIONETICS, INC. (73-3741)	302,000
MASON RES. INST. (71-2259)	556,800
MIAMI, UNIV. OF (70-2225)	108,055
MICRO. ASSOC. INC. (68-1283)	53,682
MICRO. ASSOC.,INC. (73-3728)	603,602
MURPHY FARMS (73-868)	36,167
N.Y. STATE DEPT. OF HEALTH (73-3726)	27,125
N.Y. STATE DEPT.OF HEALTH (70-2035)	58,082
NATIONAL ACADEMY OF SCIENCES #6 (64-44)	11,025
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)	33,599
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2188)	11,072
SCRIPPS CLINIC & RES. FD. (72-2042)	23,500
SIMONSEN LABS (72-725)	1,821
SIMONSEN LABS. (72-3219)	95,940
SIMONSEN LABS. (72-409)	3,374
SIMONSEN LABS. (73-816)	139,885
SIMONSEN LABS. (71-2101)	107,908
SOUTHERN ANIM.FARMS (72-732)	0
SOUTHERN ANIM.FARMS (72-2013)	49,458

TABLE II
(continued)

CONTRACT	DOLLAR
STAGE IIA: DETERMINATION OF ANTI-TUMOR ACTIVITY (CONTINUED)	
SOUTHERN ANIM.FARMS (73-863)	126,
SOUTHERN RES.INST. (71-2189)	14,
SOUTHERN RES.INST. (71-2098)	1,065,
SPARTAN RES. ANIM. INC.(73-878)	32,
SPARTAN RES. ANIM., INC.(73-858)	32,
TACONIC FARMS (73-912)	85,
TEXAS INBRED MICE (68-88)	219,
UPJOHN COMPANY (73-3707)	203,
UPJOHN COMPANY (68-1023)	13,
VALUE ENGINEERING COMPANY (73-3706)	341,
VITRO LABORATORIES (69-58)	4,
WARF INST., INC. (66-494)	177,
WASH.STATE UNIV. (72-2059)	85,
YALE UNIVERSITY (73-3711)	16,
TOTAL	10,101,

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

IVITY

CONTRACT	DOLLAR LEVEL
GE IIB: EXPERIMENTAL FORMULATION, DEVELOPMENT	
BEN VENUE LABS.,INC.(66-909)	49,966
KANSAS, UNIV. OF (72-3217)	97,997
PHILIPS ROXANE LABS.(71-2139)	0
TENNESSEE,UNIV.OF (70-2084)	18,250
WARNER-LAMBERT RESEARCH INST. (72-3250)	0
TOTAL	166,213

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEV
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS	
ARMED FORCES INST. OF PATHOLOGY (72-64)	21,54
ARS/SPRAGUE-DAWLEY (70-2044)	107,07
ARS/SPRAGUE-DAWLEY (71-2342)	84,31
ARS/SPRAGUE-DAWLEY (71-2007)	213,16
ATOMIC ENERGY COMM. (71-56)	21,00
BATTELLE MEM.INST (70-2008)	17,03
BATTELLE MEM.INST. (69-2251)	42,55
BATTELLE MEM.INST. (71-2182)	108,47
BELLAIRE ACRES (72-729)	
BELLAIRE ACRES (73-805)	12,67
BELLAIRE ACRES (73-832)	45,20
BRISTOL-MYERS CO. (69-35)	7,93
CHAS. RIV. BREEDING LABS. (73-839)	50,39
CHAS. RIV. BREEDING LABS. (73-807)	14,47
CHAS.RIV.BREEDING LABS. (72-730)	
CHAS.RIV.BREEDING LABS. (70-2043)	116,69
CHAS.RIV.BREEDING LABS. (71-2343)	67,50
CHAS.RIV.BREEDING LABS. (72-2004)	171,91
FLOW RES.ANIM.,INC. (73-838)	40,65

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
HARLAN INDUSTRIES (72-3218)	70,080
HARLAN INDUSTRIES (72-724)	0
HARLAN INDUSTRIES (73-844)	13,993
HARLAN INDUSTRIES (73-845)	48,139
HAZLETON LABS.,INC. (72-3704)	183,750
HEBREW UNIV. (71-2127)	104,940
HORTON LABS. (72-361)	218
HORTON LABS. (73-837)	31,096
IIT RESEARCH INST. (68-1021)	76,706
JACKSON LAB. (72-1171)	118,104
KANSAS, UNIV.OF (71-2010)	19,117
LAB. SUPPLY CO. (73-835)	13,634
LAB. SUPPLY CO. (73-840)	42,338
LAB.SUPPLY CO. (70-2041)	26,783
LAB.SUPPLY CO. (72-733)	0
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)	55,547
LITTLE,ARTHUR D.INC.(73-3727)	490,464
LITTLE,ARTHUR D.INC.(72-3257)	23,961
LITTLE,ARTHUR D.INC.(72-3711)	0
LITTON BIONETICS, INC. (72-3294)	92,460

TABLE II
(continued)

CONTRACT	DOLLAR LEV
STAGE IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
MASON RES. INST. (71-2259)	76,80
MICRO. ASSOC. INC. (68-1283)	48,31
MICRO. ASSOC.,INC. (73-3728)	543,24
MURPHY FARMS (73-868)	13,20
N.Y. STATE DEPT.OF HEALTH (70-2035)	21,20
NATIONAL ACADEMY OF SCIENCES #6(64-44)	4,02
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)	12,26
NOTRE DAME,UNIV.OF (72-3718)	105,11
SIMONSEN LABS (72-725)	66
SIMONSEN LABS. (72-3219)	35,02
SIMONSEN LABS. (72-409)	1,23
SIMONSEN LABS. (73-816)	51,06
SIMONSEN LABS. (71-2101)	39,39
SOUTHERN ANIM.FARMS (72-732)	
SOUTHERN ANIM.FARMS (72-2013)	52,754
SOUTHERN ANIM.FARMS (73-863)	46,046
SOUTHERN RES.INST. (66-29)	160,39
SOUTHERN RES.INST. (71-2098)	959,132
SPARTAN RES. ANIM. INC.(73-878)	11,764
SPARTAN RES. ANIM., INC.(73-858)	11,757

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
;E IIB: DETAILED EVALUATION OF NEW DRUGS (CONTINUED)	
TACONIC FARMS (73-912)	31,395
TEXAS INBRED MICE (68-88)	80,301
TULANE UNIVERSITY (73-3701)	121,485
UPJOHN COMPANY (73-3707)	81,584
UPJOHN COMPANY (68-1023)	5,332
VALUE ENGINEERING COMPANY (73-3706)	147,836
VITRO LABORATORIES (69-58)	1,100
WARF INST., INC. (66-494)	53,840
WASH.STATE UNIV. (72-2059)	31,050
TOTAL	5,301,252

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY	CONTRACT	DOLLAR LE
STAGE IIB: INITIAL ANIMAL PHARM. STUDIES		
TOTAL		

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

VITY

CONTRACT	DOLLAR LEVEL
E IIB: PROC. OF AGENTS FOR PRE-CLIN. STUDIES	
AEROJET-GEN.CORP. (68-1300)	80,797
ALDRICH CHEMICAL CO.(72-3706)	134,767
MERCK & COMPANY (72-2002)	414,771
MONSANTO RES.CORP. (73-3709)	39,770
MONSANTO RES.CORP. (67-658)	3,785
PARKE,DAVIS & CO. (72-3708)	103,408
PHARM-ECO (72-3707)	57,500
STARKS ASSOC., INC. (72-3203)	163,229
TOTAL	998,027

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LE
STAGE III: TOXICOLOGY & PHARM. IN LARGE ANIMALS	
BATTELLE MEM.INST. (71-2262)	118,0
DOW CHEMICAL CO. (72-3712)	100,0
HAZLETON LABS.,INC. (73-3212)	94,5
HAZLETON RES. ANIM.,INC. (73-9)	13,1
HAZLETON RES.ANIM., INC. (73-879)	28,9
IIT RESEARCH INST. (67-1141)	177,1
INT'L RES.&DEV.CORP (71-2264)	72,0
LITTLE,ARTHUR D.INC. (73-3727)	373,6
MARSHALL RES.ANIM., INC. (73-889)	38,8
MASON RES.INST. (70-2055)	424,4
MICRO. ASSOC. INC. (68-1283)	12,5
MICRO. ASSOC.,INC. (73-3728)	140,8
MIDWEST RES.INST. (71-2263)	100,0
MONSANTO RES.CORP. (72-3715)	149,5
SOUTH SHORE ANALYT. & RES.LAB. (64-932)	197,9
SOUTHERN RES.INST. (65-654)	292,9
SOUTHERN RES.INST. (70-2083)	145,0
STANFORD RES.INST. (71-2162)	151,9
TEXAS,UNIV.OF (66-1156)	124,0

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE III: TOXICOLOGY & PHARM. IN LARGE ANIMALS (CONTINUED)	
TRW, INC. (71-2190)	0
TRW, INC. (69-2079)	14,690
YALE UNIVERSITY (73-3740)	149,444
TOTAL	2,919,794

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LE
STAGE III: PRODUCT. & FORM. FOR CLINICAL TRIALS	
AEROJET-GEN.CORP. (68-1300)	98,7
AGRICULT.,DEPT.OF (64-1)	72,0
ALDRICH CHEMICAL CO. (72-3706)	57,7
BEN VENUE LABS.,INC. (66-909)	449,6
COLLABORATIVE RES., INC. (66-506)	67,6
DOW CHEMICAL CO. (71-2165)	200,0
MERCK & COMPANY (72-2002)	138,2
MIDWEST RES. INST. (73-3722)	163,0
MIDWEST RES.INST. (69-2113)	105,4
MONSANTO RES.CORP. (73-3709)	159,0
MONSANTO RES.CORP. (67-658)	15,1
PARKE,DAVIS & CO. (72-3708)	103,4
PHARM-ECO (72-3707)	57,5
PHILIPS ROXANE LABS. (71-2139)	
STANFORD RES. INST. (73-3742)	159,2
STANFORD RES.INST (71-2070)	86,2
STANFORD RES.INST. (73-3723)	276,4
STARKS ASSOC., INC. (72-3203)	108,8
TENNESSEE,UNIV.OF (70-2084)	54,7

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE III: PRODUCT. & FORM. FOR CLINICAL TRIALS (CONTINUED)	
WARNER-LAMBERT RESEARCH INST. (72-3250)	0
TOTAL	2,373,323

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY / CONTRACT	DOLLAR LE
STAGE IV: CLINICAL TRIALS - PHASE I	
ATOMIC ENERGY COMM. (71-59)	60,7
DUKE UNIVERSITY MEDICAL CENTER (72-2044)	26,7
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	11,7
LITTLE,ARTHUR D.INC.(72-3711)	
NATIONAL BUREAU OF STANDARDS(69-49)	36,9
TOTAL	135,7

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

IVITY

CONTRACT	DOLLAR LEVEL
GE IV: INITIAL CLIN. PHARM. STUDIES IN MAN	
CHICAGO, UNIV. OF (72-3702)	26,200
JEFFERSON MED.COLL. (70-2136)	55,000
MICRO. ASSOC. INC. (68-1283)	17,894
MICRO. ASSOC.,INC. (73-3728)	201,201
MONSANTO RES.CORP. (72-3715)	37,375
SOUTHERN RES.INST. (65-654)	39,059
STANFORD RES.INST. (71-2162)	37,995
TEXAS,UNIV.OF (66-1156)	24,800
UPJOHN COMPANY (73-3707)	138,694
UPJOHN COMPANY (68-1023)	9,064
YALE UNIVERSITY (73-3711)	7,920
TOTAL	595,202

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEV
STAGE V: CLINICAL TRIALS - PHASE II	
ALBANY MED.COLLEGE (72-2074)	34,71
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (73-3713)	108,60
ATOMIC ENERGY COMM. (71-60)	85,00
AUTOMATION INDUST., INC. (73-3717)	15,95
BOWMAN GRAY SCHOOL OF MED. (68-950)	3,99
BRANDEIS UNIV. (71-2163)	36,00
CALIFORNIA,UNIV.OF (70-2014)	5,58
CONNECTICUT, UNIV. OF (71-2181)	3,55
CONTROL DATA CORP. (67-1356)	
DUKE UNIVERSITY (70-2030)	11,34
GEORGETOWN UNIV. (71-2120)	26,29
HAZLETON LABS.,INC. (73-3708)	348,50
INDIANA UNIV.FDN. (70-2028)	9,16
KENTUCKY,UNIV.OF (67-1173)	4,60
LITTLE,ARTHUR D.INC. (72-3711)	
MAKERERE UNIV. COLLEGE COUNCIL (67-1343)	47,08
MAYO FOUNDATION (71-2185)	136,32
MAYO FOUNDATION (70-2066)	80,00
MAYO FOUNDATION (73-3735)	23,44

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
AGE V: CLINICAL TRIALS - PHASE II (CONTINUED)	
MEMORIAL HOSPITAL FOR CANCER & ALLIED DISEASES (72-2073)	0
MISSOURI,CURATORS OF THE UNIVERSITY OF(71-2323)	98,745
N.Y. STATE DEPT OF HEALTH (73-3734)	30,880
N.Y. STATE DEPT.OF HEALTH (72-2072)	40,150
NEW YORK UNIV. (67-1174)	7,125
OHIO STATE UNIV. (73-3736)	24,640
OHIO STATE UNIV. (67-1175)	4,500
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	40,500
TEXAS, UNIV. OF (73-3737)	46,780
TEXAS, UNIV. OF (73-3710)	248,660
WALTER REED ARMY MED.CENTER(64-5)	51,031
TOTAL	1,573,168

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR LEVE
STAGE VI: CLINICAL TRIALS - PHASE III	
ABCOR, INC. (70-2239)	74,846
AUTOMATION INDUST., INC. (73-3717)	39,892
BOWMAN GRAY SCHOOL OF MED. (68-950)	9,318
CALIFORNIA,UNIV.OF (70-2014)	13,040
CALIFORNIA,UNIV.OF (70-2092)	179,920
CONNECTICUT, UNIV. OF (71-2181)	8,302
CONTROL DATA CORP. (67-1356)	0
DUKE UNIVERSITY (70-2030)	26,460
GEORGETOWN UNIV. (71-2123)	254,181
GEORGETOWN UNIV. (71-2120)	65,722
HAZLETON LABS.,INC. (73-3705)	328,000
HAZLETON LABS.,INC. (72-3701)	105,400
INDIANA UNIV.FDN. (70-2028)	21,385
KENTUCKY,UNIV.OF (67-1173)	10,745
LITTLE,ARTHUR D.INC. (72-3711)	0
MAKERERE UNIV. COLLEGE COUNCIL (67-1343)	83,460
MAYO FOUNDATION (73-3735)	46,880
MICRO. ASSOC. INC. (69-2177)	39,000
N.Y. STATE DEPT OF HEALTH (73-3734)	61,760

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
GE VI: CLINICAL TRIALS - PHASE III (CONTINUED)	
NEW YORK UNIV. (67-1174)	16,622
OHIO STATE UNIV. (73-3736)	49,280
OHIO STATE UNIV. (67-1175)	10,500
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	81,000
TEXAS, UNIV. OF (73-3737)	93,560
TEXAS,UNIV.OF (71-2094)	200,000
VETERANS ADMIN. (64-4)	648,000
TOTAL	2,467,273

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

ACTIVITY

CONTRACT	DOLLAR
STAGE VI: TOX. & PHARM. STUDY LARGE ANIM. & MAN	
ASSOCIATED BIOMEDIC SYSTEMS, INC. (70-2271)	113
COLORADO STATE UNIV. (71-2192)	55
COLORADO, UNIV.OF (71-2186)	37
GEORGE WASH. UNIV. (71-2206)	65
INST. OF CANC. RES. ROYAL CANC.HOSPITAL (72-3717)	26
IST. DI RICHERCHE FARM. "MARIO NEGRI" (72-3242)	55
KANSAS, UNIV.OF (71-2184)	93
LITTLE, ARTHUR D.INC. (73-3727)	770
LITTLE, ARTHUR D.INC. (72-3711)	
LITTON BIONETICS, INC., (71-2340)	948
LITTON BIONETICS, INC., (72-2062)	111
MED.COLLEGE OF VA. (69-2266)	
MICHIGAN, REGENTS OF THE UNIVERSITY OF (72-3714)	81
N.Y. STATE DEPT. OF HEALTH (73-3726)	50
OREGON STATE UNIV. (71-2191)	
OREGON STATE UNIV. (72-3201)	69
SOUTHERN CALIF. UNIVERSITY OF (72-3241)	125
SOUTHERN RES.INST. (65-654)	58
SOUTHERN RES.INST. (70-2083)	145

TABLE II
(continued)

CONTRACT	DOLLAR LEVEL
E VI: TOX. & PHARM. STUDY LARGE ANIM. & MAN (CONTINUED)	
STANFORD RES.INST. (72-3200)	57,000
TEXAS,UNIV.OF (66-1156)	99,200
WASHINGTON,UNIV.OF (71-2193)	14,639
WISCONSIN,UNIV.OF (71-2275)	64,115
TOTAL	3,041,913

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

CTIVITY

CONTRACT	DOLLAR LEVEL
TAGE VII: CLINICAL TRIALS - ADJUVANT STUDIES	
AUTOMATION INDUST., INC. (73-3717)	23,935
CALIFORNIA,UNIV.OF (70-2092)	119,946
CLEVELAND CLIN. FD. (73-3704)	37,529
GEORGETOWN UNIV. (71-2120)	39,433
MAKERERE UNIV. COLLEGE COUNCIL (67-1343)	83,460
MAYO FOUNDATION (73-3735)	46,880
MICRO. ASSOC. INC. (69-2177)	26,000
N.Y. STATE DEPT OF HEALTH (73-3734)	61,760
OHIO STATE UNIV. (73-3736)	49,280
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)	81,000
TEXAS, UNIV. OF (73-3737)	93,560
VETERANS ADMIN. (64-4)	552,000
TOTAL	1,214,783

TABLE II
(continued)
ANALYSIS OF CONTRACTS

BY ACTIVITY

TIVITY

CONTRACT	DOLLAR LEVEL
AGE VIII: UNASSIGNED-ADMIN. & INFORM. FUNCTION	
AUTOMATION INDUST., INC. (73-3732)	142,300
HERNER INFORMATION SERVICES, INC. (72-3244)	0
INST.JULES BORDET (72-2053)	10,050
JAPANESE FD. FOR CANCER RESEARCH (72-2054)	19,775
LITTLE,ARTHUR D.INC.(72-3711)	0
NATIONAL ACADEMY OF SCIENCES #66(64-44)	0
NATIONAL ACADEMY OF SCIENCES #16(64-44)	0
THOMPSON,JOHN I.,CO.(69-2158)	38,498
TOTAL	210,623

TABLE III

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY CONTRACT

FOR FISCAL YEAR 1973

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
ABCOR, INC. (70-2239)			$ 74,846
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
AEROJET-GEN.CORP. (68-1300)			179,547
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 45% -	80,797	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 55% -	98,750	
AGRICULT.,DEPT.OF (64-1)			400,000
1XX ACQUISITION OF NEW AGENTS	- 82% -	328,000	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 18% -	72,000	
ALBANY MED.COLLEGE (72-2074)			34,714
5XX CLINICAL TRIALS - PHASE II	- 100% -		
ALDRICH CHEMICAL CO.(72-3706)			192,523
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 70% -	134,767	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 30% -	57,756	
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (73-3713)			108,600
5XX CLINICAL TRIALS - PHASE II	- 100% -		
ARIZONA BOARD OF REGENTS ARIZONA STATE UNIV. (71-2308)			46,896
1XX ACQUISITION OF NEW AGENTS	- 100% -		
ARIZONA BOARD OF REGENTS UNIV. OF ARIZONA (67-1484)			121,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
ARMED FORCES INST. OF PATHOLOGY (72-64)			21,545
2B2 DETAILED EVALUATION OF NEW DRUGS	- 100% -		
ARS/SPRAGUE-DAWLEY (70-2044)			142,761
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 25% -	35,691	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 75% -	107,070	
ARS/SPRAGUE-DAWLEY (71-2342)			112,415
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 25% -	28,104	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 75% -	84,311	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
ARS/SPRAGUE-DAWLEY (71-2007)		$	710,54
1XX ACQUISITION OF NEW AGENTS	- 13% -	92,372	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 57% -	405,010	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 30% -	213,163	
ASH STEVENS,INC. (71-2314)			69,86
1XX ACQUISITION OF NEW AGENTS	- 100% -		
ASSOCIATED BIOMEDIC SYSTEMS, INC. (70-2271)			141,60
1XX ACQUISITION OF NEW AGENTS	- 20% -	28,320	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 80% -	113,280	
ATOMIC ENERGY COMM. (71-60)			85,00
5XX CLINICAL TRIALS - PHASE II	- 100% -		
ATOMIC ENERGY COMM. (71-59)			60,78
4X1 CLINICAL TRIALS - PHASE I	- 100% -		
ATOMIC ENERGY COMM. (71-56)			21,00
2B2 DETAILED EVALUATION OF NEW DRUGS	- 100% -		
AUTOMATION INDUST., INC. (73-3732)			142,30
8XX ADMINISTRATION & INFORMATION FUNCTION	- 100% -		
AUTOMATION INDUST., INC.(73-3717)			79,78
5XX CLINICAL TRIALS - PHASE II	- 20% -	15,957	
6X1 CLINICAL TRIALS - PHASE III	- 50% -	39,892	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 30% -	23,935	
BATTELLE MEM.INST (70-2008)			74,08
1XX ACQUISITION OF NEW AGENTS	- 14% -	10,372	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	46,670	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	17,038	
BATTELLE MEM.INST. (69-2251)			185,00
1XX ACQUISITION OF NEW AGENTS	- 14% -	25,900	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	116,550	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	42,550	
BATTELLE MEM.INST. (71-2262)			118,00
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
BATTELLE MEM.INST. (71-2182)			$ 471,640
1XX ACQUISITION OF NEW AGENTS	- 14% -	66,030	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	297,133	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	108,477	
BELLAIRE ACRES (72-729)			0
1XX ACQUISITION OF NEW AGENTS	- 14% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -		
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -		
BELLAIRE ACRES (73-805)			55,120
1XX ACQUISITION OF NEW AGENTS	- 14% -	7,718	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	34,725	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	12,677	
BELLAIRE ACRES (73-832)			196,560
1XX ACQUISITION OF NEW AGENTS	- 14% -	27,520	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	123,832	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	45,208	
BEN VENUE LABS.,INC.(66-909)			499,660
2B1 EXPERIMENTAL FORMULATION, DEVELOPMENT	- 10% -	49,966	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 90% -	449,694	
BOWMAN GRAY SCHOOL OF MED.(68-950)			13,312
5XX CLINICAL TRIALS - PHASE II	- 30% -	3,994	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	9,318	
BRANDEIS UNIV. (71-2163)			36,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
BRISTOL-MYERS CO. (69-35)			793,381
1XX ACQUISITION OF NEW AGENTS	- 86% -	682,309	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 13% -	103,139	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 1% -	7,933	
BRITISH COLUMBIA, UNIV. OF (72-3223)			79,780
1XX ACQUISITION OF NEW AGENTS	- 100% -		
CALIFORNIA,UNIV.OF (70-2014)			18,629
5XX CLINICAL TRIALS - PHASE II	- 30% -	5,589	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	13,040	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
CALIFORNIA,UNIV.OF (70-2092)		$	299,86
6X1 CLINICAL TRIALS - PHASE III	- 60% -	179,920	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	119,946	
CATHOLIC MED.CTR OF BROOKLYN & QUEENS, INC.(72-3703)			184,00
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 100% -		
CENTRAL DRUG RES. INST.(P.L.480)			
1XX ACQUISITION OF NEW AGENTS	- 51% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 49% -		
CHAS. RIV. BREEDING LABS.(73-839)			219,12
1XX ACQUISITION OF NEW AGENTS	- 14% -	30,679	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	138,050	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	50,399	
CHAS. RIV. BREEDING LABS.(73-807)			62,92
1XX ACQUISITION OF NEW AGENTS	- 14% -	8,810	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	39,639	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	14,471	
CHAS.RIV.BREEDING LABS.(72-730)			
1XX ACQUISITION OF NEW AGENTS	- 14% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -		
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -		
CHAS.RIV.BREEDING LABS.(70-2043)			116,69
2B2 DETAILED EVALUATION OF NEW DRUGS	- 100% -		
CHAS.RIV.BREEDING LABS.(71-2343)			90,00
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 25% -	22,500	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 75% -	67,500	
CHAS.RIV.BREEDING LABS.(72-2004)			747,45
1XX ACQUISITION OF NEW AGENTS	- 14% -	104,645	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	470,894	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	171,913	
CHEM.ABSTR.SERVICE (65-1021)			129,40
1XX ACQUISITION OF NEW AGENTS	- 100% -		

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
CHICAGO, UNIV. OF (72-3702)			$ 26,200
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 100% -		
CLEVELAND CLIN. FD. (73-3704)			37,529
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 100% -		
COLLABORATIVE RES., INC. (71-2326)			49,343
1XX ACQUISITION OF NEW AGENTS	- 100% -		
COLLABORATIVE RES., INC. (66-506)			84,578
1XX ACQUISITION OF NEW AGENTS	- 20% -	16,916	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 80% -	67,662	
COLORADO STATE UNIV. (71-2192)			55,000
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
COLORADO,UNIV.OF (71-2186)			75,000
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 50% -	37,500	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 50% -	37,500	
CONNECTICUT, UNIV. OF (71-2181)			11,860
5XX CLINICAL TRIALS - PHASE II	- 30% -	3,558	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	8,302	
CONTROL DATA CORP. (67-1356)			0
5XX CLINICAL TRIALS - PHASE II	- 30% -		
6X1 CLINICAL TRIALS - PHASE III	- 70% -		
DELAWARE, UNIV.OF (69-2115)			20,495
1XX ACQUISITION OF NEW AGENTS	- 100% -		
DOW CHEMICAL CO. (72-3712)			100,000
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
DOW CHEMICAL CO. (71-2165)			200,039
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 100% -		
DOW CHEMICAL CO. (71-2313)			90,509
1XX ACQUISITION OF NEW AGENTS	- 100% -		

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
DUKE UNIVERSITY MEDICAL CENTER (72-2044)			$ 26,70
4X1 CLINICAL TRIALS - PHASE I	- 100% -		
DUKE UNIVERSITY (70-2030)			37,80
5XX CLINICAL TRIALS - PHASE II	- 30% -	11,340	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	26,460	
FLOW RES.ANIM.,INC. (73-838)			176,74
1XX ACQUISITION OF NEW AGENTS	- 14% -	24,745	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	111,351	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	40,652	
GEORGE WASH. UNIV. (71-2206)			65,99
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
GEORGETOWN UNIV. (71-2123)			254,18
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
GEORGETOWN UNIV. (71-2120)			131,44
5XX CLINICAL TRIALS - PHASE II	- 20% -	26,290	
6X1 CLINICAL TRIALS - PHASE III	- 50% -	65,722	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 30% -	39,433	
GEORGIA, UNIV. OF (71-2311)			36,57
1XX ACQUISITION OF NEW AGENTS	- 100% -		
HARLAN INDUSTRIES (72-3218)			146,00
1XX ACQUISITION OF NEW AGENTS	- 7% -	10,220	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 45% -	65,700	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 48% -	70,080	
HARLAN INDUSTRIES (72-724)			
1XX ACQUISITION OF NEW AGENTS	- 14% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -		
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -		
HARLAN INDUSTRIES (73-844)			60,84
1XX ACQUISITION OF NEW AGENTS	- 14% -	8,518	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	38,329	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	13,993	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
HARLAN INDUSTRIES (73-845)		$	209,300
1XX ACQUISITION OF NEW AGENTS	- 14% -	29,302	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	131,859	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	48,139	
HAWAII, UNIV.OF (67-748)			55,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
HAZLETON LABS.,INC. (73-3212)			94,550
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
HAZLETON LABS.,INC. (73-3705)			328,000
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
HAZLETON LABS.,INC. (73-3708)			348,500
5XX CLINICAL TRIALS - PHASE II	- 100% -		
HAZLETON LABS.,INC. (72-3704)			735,000
1XX ACQUISITION OF NEW AGENTS	- 23% -	169,050	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 52% -	382,200	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 25% -	183,750	
HAZLETON LABS.,INC. (72-3701)			105,400
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
HAZLETON RES. ANIM.,INC. (73-9)			13,140
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
HAZLETON RES.ANIM., INC. (73-879)			28,980
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
HEBREW UNIV. (71-2127)			104,940
2B2 DETAILED EVALUATION OF NEW DRUGS	- 100% -		
HERNER INFORMATION SERVICES, INC. (72-3244)			0
8XX ADMINISTRATION & INFORMATION FUNCTION	- 100% -		
HORTON LABS. (72-361)			950
1XX ACQUISITION OF NEW AGENTS	- 14% -	134	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	598	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	218	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
HORTON LABS. (73-837)			$ 135,20
1XX ACQUISITION OF NEW AGENTS	- 14% -	18,928	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	85,176	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	31,096	
IIT RESEARCH INST. (68-1021)			1,095,81
1XX ACQUISITION OF NEW AGENTS	- 7% -	76,709	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 86% -	942,398	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 7% -	76,706	
IIT RESEARCH INST. (67-1141)			177,15
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-2078)			85,99
1XX ACQUISITION OF NEW AGENTS	- 100% -		
ILLINOIS, BOARD OF TRUSTEES OF THE UNIVERSITY OF (72-3208)			50,37
1XX ACQUISITION OF NEW AGENTS	- 100% -		
INDIAN CANCER RES. CENTRE (P.L.480)			
1XX ACQUISITION OF NEW AGENTS	- 53% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 47% -		
INDIANA UNIV.FDN. (70-2028)			30,55
5XX CLINICAL TRIALS - PHASE II	- 30% -	9,165	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	21,385	
INST. OF CANC. RES. ROYAL CANC.HOSPITAL (72-3717)			26,77
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
INST.JULES BORDET (72-2053)			15,00
1XX ACQUISITION OF NEW AGENTS	- 33% -	4,950	
8XX ADMINISTRATION & INFORMATION FUNCTION	- 67% -	10,050	
INT'L RES.&DEV.CORP (71-2264)			72,01
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
IST. DI RICHERCHE FARM. "MARIO NEGRI" (72-3242)			55,00
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
IST. DI RICHERCHE FARM. 'MARIO NEGRI' (73-3720)		$	124,192
1XX ACQUISITION OF NEW AGENTS	- 20% -	24,839	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 80% -	99,353	
JACKSON LAB. (72-1171)			218,712
1XX ACQUISITION OF NEW AGENTS	- 6% -	13,124	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 40% -	87,484	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 54% -	118,104	
JAPANESE FD. FOR CANCER RESEARCH (72-2054)			56,500
1XX ACQUISITION OF NEW AGENTS	- 20% -	11,300	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 25% -	14,125	
4X1 CLINICAL TRIALS - PHASE I	- 20% -	11,300	
8XX ADMINISTRATION & INFORMATION FUNCTION	- 35% -	19,775	
JEFFERSON MED.COLL. (70-2136)			55,000
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 100% -		
KANSAS, UNIV. OF (72-3217)			97,997
2B1 EXPERIMENTAL FORMULATION, DEVELOPMENT	- 100% -		
KANSAS, UNIV.OF (71-2010)			83,118
1XX ACQUISITION OF NEW AGENTS	- 14% -	11,637	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	52,364	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	19,117	
KANSAS,UNIV.OF (71-2184)			93,484
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
KENTUCKY,UNIV.OF (67-1173)			15,350
5XX CLINICAL TRIALS - PHASE II	- 30% -	4,605	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	10,745	
LAB. SUPPLY CO. (73-835)			59,280
1XX ACQUISITION OF NEW AGENTS	- 14% -	8,300	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	37,346	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	13,634	
LAB. SUPPLY CO. (73-840)			184,080
1XX ACQUISITION OF NEW AGENTS	- 14% -	25,772	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	115,970	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	42,338	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
LAB.SUPPLY CO. (70-2041)			$ 116,44
1XX ACQUISITION OF NEW AGENTS	- 14% -	16,304	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	73,362	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	26,783	
LAB.SUPPLY CO. (72-733)			
1XX ACQUISITION OF NEW AGENTS	- 14% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -		
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -		
LEO GOODWIN INST. FOR CANCER RESEARCH (73-3729)			241,51
1XX ACQUISITION OF NEW AGENTS	- 14% -	33,813	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	152,152	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	55,547	
LITTLE,ARTHUR D.INC.(73-3727)			2,335,54
1XX ACQUISITION OF NEW AGENTS	- 2% -	46,713	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 28% -	653,951	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 21% -	490,464	
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 16% -	373,687	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 33% -	770,729	
LITTLE,ARTHUR D.INC.(72-3257)			119,80
1XX ACQUISITION OF NEW AGENTS	- 13% -	15,576	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 67% -	80,270	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 20% -	23,961	
LITTLE,ARTHUR D.INC.(72-3711)			
2B2 DETAILED EVALUATION OF NEW DRUGS	- 10% -		
4X1 CLINICAL TRIALS - PHASE I	- 24% -		
5XX CLINICAL TRIALS - PHASE II	- 3% -		
6X1 CLINICAL TRIALS - PHASE III	- 15% -		
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 7% -		
8XX ADMINISTRATION & INFORMATION FUNCTION	- 41% -		
LITTON BIONETICS, INC.,(71-2340)			1,355,17
1XX ACQUISITION OF NEW AGENTS	- 30% -	406,552	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 70% -	948,621	
LITTON BIONETICS, INC. (72-3294)			402,00
1XX ACQUISITION OF NEW AGENTS	- 14% -	56,280	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	253,260	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	92,460	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
LITTON BIONETICS, INC., (72-2062)			$ 111,400
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
LITTON BIONETICS, INC. (73-3741)			302,000
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 100% -		
MAKERERE UNIV. COLLEGE COUNCIL (67-1343)			214,000
5XX CLINICAL TRIALS - PHASE II	- 22% -	47,080	
6X1 CLINICAL TRIALS - PHASE III	- 39% -	83,460	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 39% -	83,460	
MARSHALL RES.ANIM., INC. (73-889)			38,880
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
MASON RES. INST. (71-2259)			640,000
1XX ACQUISITION OF NEW AGENTS	- 1% -	6,400	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 87% -	556,800	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 12% -	76,800	
MASON RES.INST. (70-2055)			424,457
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
MAYO FOUNDATION (71-2185)			136,325
5XX CLINICAL TRIALS - PHASE II	- 100% -		
MAYO FOUNDATION (70-2066)			80,000
5XX CLINICAL TRIALS - PHASE II	- 100% -		
MAYO FOUNDATION (73-3735)			117,200
5XX CLINICAL TRIALS - PHASE II	- 20% -	23,440	
6X1 CLINICAL TRIALS - PHASE III	- 40% -	46,880	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	46,880	
MED.COLLEGE OF VA. (69-2266)			0
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
MEMORIAL HOSPITAL FOR CANCER & ALLIED DISEASES (72-2073)			0
5XX CLINICAL TRIALS - PHASE II	- 100% -		
MERCK & COMPANY (72-2002)			553,027
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 75% -	414,771	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 25% -	138,256	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
MIAMI, UNIV. OF (70-2225)		$	180,09
1XX ACQUISITION OF NEW AGENTS	- 40% -	72,037	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 60% -	108,055	
MICHIGAN, REGENTS OF THE UNIV. OF (69-2004)			50,00
1XX ACQUISITION OF NEW AGENTS	- 100% -		
MICHIGAN,REGENTS OF THE UNIVERSITY OF (72-3714)			81,20
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
MICHIGAN,STATE OF, DEPT. OF PUBLIC HEALTH (73-3702)			45,11
1XX ACQUISITION OF NEW AGENTS	- 100% -		
MICRO. ASSOC. INC.(69-2177)			65,00
6X1 CLINICAL TRIALS - PHASE III	- 60% -	39,000	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	26,000	
MICRO. ASSOC. INC. (68-1283)			178,94
1XX ACQUISITION OF NEW AGENTS	- 26% -	46,530	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 30% -	53,682	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 27% -	48,314	
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 7% -	12,526	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 10% -	17,894	
MICRO. ASSOC.,INC. (73-3728)			2,012,01
1XX ACQUISITION OF NEW AGENTS	- 26% -	523,126	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 30% -	603,602	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 27% -	543,243	
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 7% -	140,840	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 10% -	201,201	
MIDWEST RES. INST. (73-3743)			331,16
1XX ACQUISITION OF NEW AGENTS	- 100% -		
MIDWEST RES. INST. (73-3722)			163,06
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 100% -		
MIDWEST RES.INST. (71-2263)			100,00
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
MIDWEST RES.INST. (69-2113)			$ 105,412
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 100% -		
MIDWEST RES.INST. (65-94)			99,989
1XX ACQUISITION OF NEW AGENTS	- 100% -		
MISSOURI,CURATORS OF THE UNIVERSITY OF(71-2323)			98,745
5XX CLINICAL TRIALS - PHASE II	- 100% -		
MONSANTO RES.CORP. (73-3709)			198,850
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 20% -	39,770	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 80% -	159,080	
MONSANTO RES.CORP. (72-3715)			186,876
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 80% -	149,501	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 20% -	37,375	
MONSANTO RES.CORP. (67-658)			18,924
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 20% -	3,785	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 80% -	15,139	
MURPHY FARMS (73-868)			57,408
1XX ACQUISITION OF NEW AGENTS	- 14% -	8,038	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	36,167	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	13,203	
N.Y. STATE DEPT OF HEALTH (73-3734)			154,400
5XX CLINICAL TRIALS - PHASE II	- 20% -	30,880	
6X1 CLINICAL TRIALS - PHASE III	- 40% -	61,760	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	61,760	
N.Y. STATE DEPT. OF HEALTH(73-3726)			77,500
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 35% -	27,125	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 65% -	50,375	
N.Y. STATE DEPT.OF HEALTH (72-2072)			40,150
5XX CLINICAL TRIALS - PHASE II	- 100% -		
N.Y. STATE DEPT.OF HEALTH (70-2035)			92,194
1XX ACQUISITION OF NEW AGENTS	- 14% -	12,908	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	58,082	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	21,204	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
NATIONAL ACADEMY OF SCIENCES #66(64-44)		$	(
8XX ADMINISTRATION & INFORMATION FUNCTION	- 100% -		
NATIONAL ACADEMY OF SCIENCES #16(64-44)			(
8XX ADMINISTRATION & INFORMATION FUNCTION	- 100% -		
NATIONAL ACADEMY OF SCIENCES #6(64-44)			17,50(
1XX ACQUISITION OF NEW AGENTS	- 14% -	2,450	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	11,025	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	4,025	
NATIONAL BUREAU OF STANDARDS(69-49)			36,948
4X1 CLINICAL TRIALS - PHASE I	- 100% -		
NEW JERSEY COLLEGE OF MED.& DENTISTRY (72-3211)			53,333
1XX ACQUISITION OF NEW AGENTS	- 14% -	7,468	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	33,599	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	12,266	
NEW YORK UNIV. (67-1174)			23,747
5XX CLINICAL TRIALS - PHASE II	- 30% -	7,125	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	16,622	
NOTRE DAME,UNIV.OF (72-3718)			105,116
2B2 DETAILED EVALUATION OF NEW DRUGS	- 100% -		
OHIO STATE UNIV. (73-3736)			123,200
5XX CLINICAL TRIALS - PHASE II	- 20% -	24,640	
6X1 CLINICAL TRIALS - PHASE III	- 40% -	49,280	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	49,280	
OHIO STATE UNIV. (67-1175)			15,000
5XX CLINICAL TRIALS - PHASE II	- 30% -	4,500	
6X1 CLINICAL TRIALS - PHASE III	- 70% -	10,500	
OREGON STATE UNIV. (71-2191)			(
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
OREGON STATE UNIV. (72-3201)			69,013
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
PARKE,DAVIS & CO. (72-3708)			$ 206,816
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 50% -	103,408	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 50% -	103,408	
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (73-3719)			492,390
1XX ACQUISITION OF NEW AGENTS	- 100% -		
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2188)			11,072
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 100% -		
PENNSYLVANIA, TRUSTEES OF THE UNIVERSITY OF (71-2187)			50,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
PENNWALT CORP. (71-2309)			60,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
PHARM-ECO (72-3707)			115,000
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 50% -	57,500	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 50% -	57,500	
PHILIPS ROXANE LABS. (71-2139)			0
2B1 EXPERIMENTAL FORMULATION, DEVELOPMENT	- 20% -		
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 80% -		
POMONA COLLEGE (73-3703)			38,940
1XX ACQUISITION OF NEW AGENTS	- 100% -		
RESEARCH FD. OF STATE UNIV. OF NEW YORK (73-3715)			202,500
5XX CLINICAL TRIALS - PHASE II	- 20% -	40,500	
6X1 CLINICAL TRIALS - PHASE III	- 40% -	81,000	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	81,000	
RESEARCH TRIANGLE INST. (69-2019)			139,951
1XX ACQUISITION OF NEW AGENTS	- 100% -		
RHODE ISLAND, UNIV. OF (71-2312)			39,450
1XX ACQUISITION OF NEW AGENTS	- 100% -		
SCRIPPS CLINIC & RES. FD. (72-2042)			47,000
1XX ACQUISITION OF NEW AGENTS	- 50% -	23,500	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 50% -	23,500	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
SHEEHAN INST.FOR RES. (72-3719)			$ 52,24
1XX ACQUISITION OF NEW AGENTS	- 100% -		
SIMONSEN LABS (72-725)			2,89
1XX ACQUISITION OF NEW AGENTS	- 14% -	406	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	1,821	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	664	
SIMONSEN LABS. (72-3219)			152,28
1XX ACQUISITION OF NEW AGENTS	- 14% -	21,321	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	95,940	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	35,026	
SIMONSEN LABS. (72-409)			5,35
1XX ACQUISITION OF NEW AGENTS	- 14% -	751	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	3,374	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	1,232	
SIMONSEN LABS. (73-816)			222,04
1XX ACQUISITION OF NEW AGENTS	- 14% -	31,086	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	139,885	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	51,069	
SIMONSEN LABS. (71-2101)			171,28
1XX ACQUISITION OF NEW AGENTS	- 14% -	23,980	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	107,908	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	39,395	
SOUTH SHORE ANALYT. & RES.LAB.(64-932)			197,99
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
SOUTHERN ANIM.FARMS (72-732)			
1XX ACQUISITION OF NEW AGENTS	- 14% -		
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -		
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -		
SOUTHERN ANIM.FARMS (72-2013)			109,90
1XX ACQUISITION OF NEW AGENTS	- 7% -	7,696	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 45% -	49,458	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 48% -	52,754	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT	DOLLAR LEVEL
SOUTHERN ANIM.FARMS (73-863)		$ 200,200
1XX ACQUISITION OF NEW AGENTS	- 14% -	28,028
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	126,126
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	46,046
SOUTHERN CALIF. UNIVERSITY OF (72-3241)		125,000
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -	
SOUTHERN RES.INST. (73-3712)		561,000
1XX ACQUISITION OF NEW AGENTS	- 100% -	
SOUTHERN RES.INST. (65-654)		390,590
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 75% -	292,943
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 10% -	39,059
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 15% -	58,588
SOUTHERN RES.INST. (66-29)		320,780
1XX ACQUISITION OF NEW AGENTS	- 50% -	160,390
2B2 DETAILED EVALUATION OF NEW DRUGS	- 50% -	160,390
SOUTHERN RES.INST. (71-2189)		14,000
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 100% -	
SOUTHERN RES.INST. (70-2083)		290,000
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 50% -	145,000
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 50% -	145,000
SOUTHERN RES.INST. (71-2098)		2,131,407
1XX ACQUISITION OF NEW AGENTS	- 5% -	106,573
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 50% -	1,065,702
2B2 DETAILED EVALUATION OF NEW DRUGS	- 45% -	959,132
SOUTHERN RES.INST. (71-2021)		76,000
1XX ACQUISITION OF NEW AGENTS	- 100% -	
SPARTAN RES. ANIM. INC. (73-878)		51,150
1XX ACQUISITION OF NEW AGENTS	- 14% -	7,162
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	32,224
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	11,764

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
SPARTAN RES. ANIM., INC. (73-858)		$	51,12
1XX ACQUISITION OF NEW AGENTS	- 14% -	7,158	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	32,205	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	11,757	
STANFORD RES. INST. (73-3742)			398,16
1XX ACQUISITION OF NEW AGENTS	- 60% -	238,896	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 40% -	159,264	
STANFORD RES.INST (71-2070)			215,65
1XX ACQUISITION OF NEW AGENTS	- 60% -	129,393	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 40% -	86,261	
STANFORD RES.INST. (71-2162)			189,97
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 80% -	151,984	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 20% -	37,995	
STANFORD RES.INST. (73-3723)			276,47
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 100% -		
STANFORD RES.INST. (72-3200)			57,00
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
STARKS ASSOC., INC. (72-3203)			272,04
2B4 PROC. OF AGENTS FOR PRE-CLIN. STUDIES	- 60% -	163,229	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 40% -	108,819	
STARKS,C.P.,INC. (72-3705)			135,50
1XX ACQUISITION OF NEW AGENTS	- 100% -		
TACONIC FARMS (73-912)			136,50
1XX ACQUISITION OF NEW AGENTS	- 14% -	19,110	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	85,995	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	31,395	
TENNESSEE,UNIV.OF (70-2084)			73,00
2B1 EXPERIMENTAL FORMULATION, DEVELOPMENT	- 25% -	18,250	
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 75% -	54,750	
TEXAS INBRED MICE (68-88)			349,13
1XX ACQUISITION OF NEW AGENTS	- 14% -	48,880	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	219,956	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	80,301	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
EXAS, UNIV. OF (73-3737)		$	233,900
5XX CLINICAL TRIALS - PHASE II	- 20% -	46,780	
6X1 CLINICAL TRIALS - PHASE III	- 40% -	93,560	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 40% -	93,560	
EXAS, UNIV. OF (73-3710)			248,660
5XX CLINICAL TRIALS - PHASE II	- 100% -		
EXAS,UNIV.OF (71-2094)			200,000
6X1 CLINICAL TRIALS - PHASE III	- 100% -		
EXAS,UNIV.OF (66-1156)			248,000
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 50% -	124,000	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 10% -	24,800	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 40% -	99,200	
HOMPSON,JOHN I.,CO.(69-2158)			38,498
8XX ADMINISTRATION & INFORMATION FUNCTION	- 100% -		
RW, INC. (71-2190)			0
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
RW, INC. (69-2079)			14,690
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
ULANE UNIVERSITY (73-3701)			121,485
2B2 DETAILED EVALUATION OF NEW DRUGS	- 100% -		
PJOHN COMPANY (73-3707)			815,849
1XX ACQUISITION OF NEW AGENTS	- 48% -	391,610	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 25% -	203,961	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 10% -	81,584	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 17% -	138,694	
PJOHN COMPANY (72-3709)			35,978
1XX ACQUISITION OF NEW AGENTS	- 100% -		
PJOHN COMPANY (68-1023)			53,320
1XX ACQUISITION OF NEW AGENTS	- 48% -	25,595	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 25% -	13,329	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 10% -	5,332	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 17% -	9,064	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
UTAH, UNIV. OF (72-3710)			$ 60,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
VALUE ENGINEERING COMPANY (73-3706)			568,600
1XX ACQUISITION OF NEW AGENTS	- 14% -	79,604	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 60% -	341,160	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 26% -	147,836	
VETERANS ADMIN. (64-4)			1,200,000
6X1 CLINICAL TRIALS - PHASE III	- 54% -	648,000	
7XX CLINICAL TRIALS - ADJUVANT STUDIES	- 46% -	552,000	
VIRGINIA, UNIV.OF (71-2099)			129,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
VITRO LABORATORIES (69-58)			6,475
1XX ACQUISITION OF NEW AGENTS	- 16% -	1,037	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 67% -	4,338	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 17% -	1,100	
WALTER REED ARMY MED.CENTER(64-5)			51,031
5XX CLINICAL TRIALS - PHASE II	- 100% -		
WARF INST., INC. (66-494)			538,402
1XX ACQUISITION OF NEW AGENTS	- 57% -	306,890	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 33% -	177,672	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 10% -	53,840	
WARF INST.,INC. (67-747)			0
1XX ACQUISITION OF NEW AGENTS	- 100% -		
WARNER-LAMBERT RESEARCH INST. (72-3250)			0
2B1 EXPERIMENTAL FORMULATION, DEVELOPMENT	- 20% -		
3X2 PRODUCT. & FORM. FOR CLINICAL TRIALS	- 80% -		
WASH.STATE UNIV. (72-2059)			135,000
1XX ACQUISITION OF NEW AGENTS	- 14% -	18,900	
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 63% -	85,050	
2B2 DETAILED EVALUATION OF NEW DRUGS	- 23% -	31,050	

TABLE III
(continued)

CONTRACTOR/FUNCTIONS	PERCENT OF EFFORT		DOLLAR LEVEL
SHINGTON,UNIV.OF (71-2193)			$ 14,639
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 100% -		
IZMANN INST.OF SCIENCE(P.L.480)			0
1XX ACQUISITION OF NEW AGENTS	- 100% -		
SCONSIN,BOARD OF REGENTS OF THE UNIVERSITY OF (73-3733)			30,691
1XX ACQUISITION OF NEW AGENTS	- 100% -		
SCONSIN,UNIV.OF (71-2275)			85,487
1XX ACQUISITION OF NEW AGENTS	- 25% -	21,372	
6X2 TOX. & PHARM. STUDY LARGE ANIM. & MAN	- 75% -	64,115	
LE UNIVERSITY (71-2339)			60,000
1XX ACQUISITION OF NEW AGENTS	- 100% -		
LE UNIVERSITY (73-3740)			149,444
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	- 100% -		
LE UNIVERSITY (73-3711)			24,000
2AX DETERMINATION OF ANTI-TUMOR ACTIVITY	- 67% -	16,080	
4X2 INITIAL CLIN. PHARM. STUDIES IN MAN	- 33% -	7,920	

TABLE IV
DESCRIPTION OF CONTRACTS
IN THE
DIVISION OF CANCER TREATMENT
PROGRAM

ABCOR, INC. (NIH-NCI-C-70-2239)

This contract provides feasibility investigations and engineering developments to significantly improve the yields of human blood cells, particularly leukocytes, from hematologically normal donors, (a) through modifications of the NCI-IBM Blood Cell Separator or its operational techniques, or (b) through entirely new systems of procurement based upon different principles of blood cell separation such as centrifugal elutriation, filtration leukapheresis, etc.

AEROJET-GENERAL CORPORATION (PH43-NCI-C-68-1300)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

AGRICULTURE, U. S. DEPARTMENT OF (TRANSFER OF FUNDS - NCI-C-64-1)

Under the transfer of funds agreement with the U.S.D.A., the New Crops Research Division (now called the Medicinal Plant Resources Laboratory) collects approximately 8,000 new plant materials per year for screening. Plant samples are obtained from all parts of the world.

The plants collected by the U.S.D.A. are shipped to the extraction contractor, WARF Institute, Inc. As confirmed active plants appear, the U.S.D.A. recollects in large quantity, on a priority based on the needs of the Division of Cancer Treatment Program, at the rate of about 220 recollections per year for shipment to the fractionating chemists. Very large recollections are made, as requested by Drug Research and Development, for the preparation of clinical quantities of drugs.

ALBANY MEDICAL COLLEGE (NIH-NCI-C-72-2074)

This contract was designed to evaluate the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival. Case accrual has been over 28 patients on a study comparing CCNU and 5-azacytidine with a total of 58 from both Albany and Roswell Park. Regressions with 5-azacytidine have been seen in 2/21 patients evaluable and with CCNU in 2/19.

ALDRICH CHEMICAL COMPANY (NIH-NCI-C-72-3706)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in

ALDRICH CHEMICAL COMPANY (NIH-NCI-C-72-3706) Continued

the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range requiring pilot plant facilities.

AMERICAN NATIONAL RED CROSS (NIH-70-2005)

The Red Cross supplied the Medical Oncology area and other areas of the National Cancer Institute with 120-150 units of platelet concentrates (fresh) per week for experimental use in the clinical wards. This contract terminated September 1972.

ANTEC CORPORATION (NIH-72-C-467)

This contract provided 150 beagle hounds for toxicologic assays. These animals were raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals were supplied by the Contractor. This contract terminated January 1973.

ARIZONA BOARD OF REGENTS (ARIZONA STATE UNIVERSITY)(NIH-NCI-C-71-2308)

The major objective of this contract is the preparation, from insects, marine animal and plant specimens, of extracts suitable for bioassay in the NCI anti-tumor screen. The raw natural product specimens are obtained in most instances by the Contractor with emphasis on animal and marine sources. However, on occasion, the specimens for extraction are supplied from other sources. The extracts are prepared in accordance with procedures acceptable to NCI and in amounts suitable for routine screening. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation.

ARIZONA BOARD OF REGENTS (ARIZONA, UNIVERSITY OF) (PH43-NCI-C-67-1484)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Recent promising results have been obtained from the extraction of plants collected in Greece, Hawaii and Arizona. Further fractionation is being carried out to isolate the active components. During the last year, a KB cell culture unit has been added to expedite the fractionation work.

ARIZONA, UNIVERSITY OF (NO1-CM-33713)

The initial year of this contract (begun November 10, 1972) is devoted to the development of a staging system for patients with multiple myeloma. The rationale for this proposed staging system is based on detailed studies by the Principal Investigator of a procedure for determing total myeloma cell number in patients. The proposed system would incorporate available detailed clinical and laboratory data and measurements in conjunction with tumor cell number to derive a means for staging myeloma patients. This system will then be tried prospectively to determine if the desired correlations with actual tumor cell measurements hold true. Such a system can then be used for evaluation by

ARIZONA, UNIVERSITY OF (NO1-CM-33713) Continued

clinical parameters of the number of tumor cells remaining following therapy without the necessity for carrying out the detailed and involved procedures for actual measurement of this number.

ARMED FORCES INSTITUTE OF PATHOLOGY (NIH-NCI-C-72-64)

This is an interagency agreement for the preparation of antihuman rabbit antilymphocyte serum.

ARS/SPRAGUE-DAWLEY (NIH-NCI-C-70-2044)

This rodent production center contract supports a production effort designed to furnish approximately 80,000-90,000 animals annually. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

ARS/SPRAGUE-DAWLEY (NIH-NCI-C-71-2007)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in closely controlled environments. The overall workscope comprises four distinct tasks identified as follows:

Task 1 provides inbred, outbred and hybrid rodents in modified conventional environments.

Task 2 provides Syrian golden hamsters with defined but non-pathogenic organisms. A small percentage of the effort is devoted to the development of germfree hamsters and gerbils.

Task 3 provides inbred and outbred laboratory strains of rats as germfree and/or with defined microflora.

Task 4 provides various germfree inbred, outbred and hybrids of inbred rodents, and the production of counterparts of the various species with defined microflora.

ARS/SPRAGUE-DAWLEY (NIH-NCI-C-71-2342)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of AKR mice.

ARS/SPRAGUE-DAWLEY (NIH-72-C-359)

This contract furnished approximately 312,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals were furnished from genetic center and/or rodent production colonies. This contract terminated March 1973.

ASH STEVENS, INC. (NIH-NCI-C-71-2314)

The objective of this contract is the synthesis of potential antitumor oxygen and/or sulfur heterocyclic compounds. The relatively unexplored areas of 1,2,4-trithiolanes and cyclic disulfides are to be examined as carriers for sulfur and oxygen heterocyclics, metabolically active functions, and antimetabolites. Advantage will be taken of any leads which develop during the course of the work to extend the syntheses into new potentially active structural areas.

ASSOCIATED BIOMEDIC SYSTEMS (NIH-NCI-C-70-2271)

The purposes of this contract are: (1) to supply normal human blood lymphocytes, both untreated and stimulated with phytohemagglutinin; (2) to produce and evaluate human myeloid colony-stimulating factor(s) by "conditioning" media with various cells and then testing such media for "factor" activity; (3) to perform various services regarding culture of human cell lines such as (a) mass production of "interesting" lines (e.g., cells containing RNA-directed DNA polymerase or cell producing "factors"), and (b) ridding cell lines of contaminating mycoplasma by various techniques; (4) procurement of sera and cells from patients with tumors, and of hematopoietic tissue (e.g., bone marrow and spleen) from normal individuals. About 250 billion lymphocyte were provided last year. Certain cell-free "conditioned" media have been identified as rich sources of colony-stimulating factor. At least one cell line has been freed of detectable mycoplasma recently.

ATOMIC ENERGY COMMISSION (NCI-CR-71-56)

The principal objective of this project is to study the cell cycle stage specificity of clinical drugs and drugs in development toward clinical trial. Drugs are evaluated for their ability to inhibit cells in G_1, G_2 and M as well as in the S phase of their life cycle. The importance of inhibitors of phases of the cell cycle other than S stems from the need to develop drugs which may be specifically cytotoxic to non-proliferating but viable and clonogenic tumor cells. The *in vitro* methodologies used were developed by the Contractor.

ATOMIC ENERGY COMMISSION (NCI-CR-71-59)

This contract is for support for a joint effort of the AEC Cooperative Group to study localization and to determine the usefulness of Gallium-67 in Hodgkin's disease and other lymphomas, as well as lung cancer. The immediate objective is designed to determine the value of this agent as an additional diagnostic aid for staging and determining the effectiveness and completeness of treatment. To date, 16 cooperating institutions have followed a single protocol for injecting and scanning patients with Hodgkin's and other lymphoma. Standardized procedures are being developed; instrumentation, encoding and observations made. Detailed data analysis programs are being applied and correlations with histologic types are being done.

ATOMIC ENERGY COMMISSION (NCI-CR-71-60)

This contract involves the study of potential biologic markers for cancer in body fluids by means of high resolution, high pressure ion exchange

ATOMIC ENERGY COMMISSION (NCI-CR-71-60) Continued

chromatography. Initial efforts have focused on minor and methylated nucleic acid derivatives (tRNA catabolic end products) in urine. Sensitive analytical tools utilized in addition to high pressure chromatography include gas chromatography, gas chromatography-mass spectrometry and high- and low-resolution mass spectrometry. Preliminary quantitative results indicate elevated levels for specific nucleosides in the urine of approximately 60% of the cancer patients studied. Future efforts will include the evaluation of basic materials in urine as potential biologic markers utilizing similar analytical techniques.

AUTOMATION INDUSTRIES, INC. (NIH-NCI-C-73-3717)

This contract is designed to provide the Division of Cancer Treatment with the preparation of clinical data for computer processing. The Contractor will extract data from medical records and other clinical sources and assist in the maintenance of existing computer files and the generation of computer retrievals and reports.

AUTOMATION INDUSTRIES, INC. (NO1-CM-33732)

This professional services contract was established to provide the necessary administrative and technical support to the Office of the Director, Division of Cancer Treatment. The primary function is the coordination and technical skills involved with the organization of conferences, graphic support and editorial services.

BATTELLE MEMORIAL INSTITUTE (NIH-NCI-C-69-2251)

The Contractor maintains a genetic production center for inbred rodents which provides breeding animals for large-scale laboratory rodent production colonies and limited numbers for testing and research programs. The profile of categories of these animals includes ten strains.

BATTELLE MEMORIAL INSTITUTE (NIH-NCI-C-70-2008)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of *Salmonella spp.* and *Pseudomonas spp.* in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples are screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques and the elucidation of salmonella inhibitory substances associated with certain microorganisms.

BATTELLE MEMORIAL INSTITUTE (NIH-NCI-C-71-2182)

Battelle Memorial Institute conducts *in vivo* testing of new synthetic materials and natural products of both plant and animal origin, as well as the secondary evaluation of active materials. This testing is being conducted at a rate of approximately 37,000 tests per annum. As per the current Drug Research and Development protocols, new synthetic materials are tested in the Leukemia L1210

BATTELLE MEMORIAL INSTITUTE (NIH-NCI-C-71-2182) Continued

tumor system and in the B16 Melanoma for specific materials. New natural products are tested in the P-388 Leukemia, with those displaying efficacy in this system being scheduled in the L1210 Leukemia. The tumor assay systems may be changed by the Project Officer dependent upon the needs of the DR&D program. A tumor bank is maintained with the tumors stored being available to DR&D. Schedule dependency studies to determine the optimal dose, schedule, route of administration and host toxicity are conducted on active materials. At the Project Officer's request, methodologic studies are carried out.

BATTELLE MEMORIAL INSTITUTE (NIH-NCI-C-71-2262)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

BELLAIRE ACRES (NIH-73-C-805) (formerly NIH-72-C-729)

This procurement contract is designed to furnish yearly 104,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for Drug Research and Development contrac studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to elminate or control the incidence of pseudomonads.

BELLAIRE ACRES (NIH-73-C-832) (formerly NIH-72-C-376)

This contract furnishes approximately 364,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybri mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

BEN VENUE LABORATORIES, INC. (PH43-NCI-C-66-909)

This contract is designed to provide a pharmaceutical facility for the develop ment and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile parenteral products. Specifically, the Contractor performs the following duties:

1. Solubility and stability studies on chemical agents prior to development of a specific dosage form;

2. Development and production of parenteral dosage forms for clinical evaluation;

BEN VENUE LABORATORIES, INC. (PH43-NCI-C-66-909) Continued

3. Stability surveillance of all dosage forms prepared.

All projects are initiated by the Project Officer for the contract.

BOWMAN GRAY SCHOOL OF MEDICINE (PH43-NCI-C-68-950)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy, including chemotherapy, in accordance with protocols of the Brain Tumor Study Group so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

BRANDEIS UNIVERSITY (NIH-NCI-C-71-2163)

The objective of this contract is to develop radioimmunoassays to detect and measure quantitatively specific methylated nucleic acid bases (constituents of tRNA) in blood serum of cancer patients. Difficulties have been encountered in working out as sensitive a procedure as desired. Results to date for 7-methylguanine and N_2,N_2-dimethylguanosine do not indicate their presence in serum. The remainder of this year's contract will be devoted to similar studies to assay serum pseudouridine. On the basis of the results to date, further continuation of this contract after this year is not contemplated.

BRISTOL-MYERS COMPANY (NIH-NCI-C-69-35)

This contract covers a comprehensive effort to obtain new antitumor agents from fermentation broths or other natural products. It includes (1) the preparation of broths, or other natural products, for screening against *in vivo* systems specified by NCI, (2) a rodent tumor laboratory for *in vivo* screening and also testing of fractionation and other follow-up studies, (3) the isolation work required to obtain purified agents, (4) development and use of *in vitro* correlative assays for more rapid follow-up of such samples, (5) production of large quantities of agents approved for clinical trials, and (6) the preparation of appropriate dosage forms of such agents.

BRITISH COLUMBIA, UNIVERSITY OF (NIH-NCI-C-72-3223)

The major objective of this contract is to develop a general and versatile synthesis of the highly complex and clinically effective dimeric vinca alkaloids, vincristine and vinblastine. The synthesis of some related analogs and derivatives and the preparation of radiolabeled vincristine is being undertaken. In addition, the contract provides for the final isolation, purification and identification of metabolites obtained from samples collected by investigators specified by NCI after *in vivo* administration of the radiolabeled vincristine.

CALIFORNIA, UNIVERSITY OF (NIH-NCI-C-70-2014)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy,

CALIFORNIA, UNIVERSITY OF (NIH-NCI-C-70-2014) Continued

including chemotherapy, in accordance with protocols of the Brain Tumor Study Group so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

CALIFORNIA, UNIVERSITY OF (NIH-NCI-C-70-2092)

This contract is designed to provide HL-A typing on all NCI patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service in Baltimore. Trays used in the analysis of antigen specificities will be updated with new sera as frequently as necessary to maintain high levels of reproducibility and reliability according to current scientific information in the field of HL-A typing. To date almost 400 cell typings and serum analyses have been done per month. The Contractor has provided computer programs so that remote searches can be made from the NCI plasmapheresis facility. This enlarged donor pool is now more completely HL-A typed and gives more available HL-A matched donors for platelet transfusion.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (NIH-NCI-C-72-3703)

This contract was initiated to provide an experimental model for human breast cancer therapy. The assay system utilized is the CD_8F_1 spontaneous and first generation transplanted tumor. During this first contract year, drugs have been tested against the first generation transplanted tumor. The drugs being tested are those specified by the Division of Cancer Treatment staff and are those with established clinicalactivity and candidates for clinical trial. The contract is designed to provide a human tumor model which will enable us to (1) test drugs to determine those with specificity against breast carcinoma, (2) study the influence of treatment schedule on "slow growing" tumors, and (3) study drug combinations as well as drugs combined with surgery and immunotherapy. This contract complements an established program for chemotherapy and immunology at the Medical Center.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - Agreement No. NIH-01-004-1)

The Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by Drug Research and Development, and fractionates the active extracts. During the past four years the budget was increased to provide for (1) synthesis of compounds of chemotherapeutic interest, and (2) setting up an antitumor screening laboratory to provide in-house testing capability. Several compounds have been received by DR&D and limited in-house testing has been achieved.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-NCI-C-70-2043)

This rodent production center contract supports a production effort designed to furnish approximately 60,000-70,000 animals annually. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-NCI-C-71-2343)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-NCI-C-72-2004)

This primary genetic center has as its objectives the development of germfree foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germfree isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in jacket isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. A colony of agoutis (Dasyprocta agouti) is maintained in order to furnish serum for L-asparaginase extraction. Task 1 is concerned with the production of germfree rodents, counterparts with defined microbial flora and large-scale production; Task 2 in Calco, Italy, produces rodents in bio-containment environments; and Tasks 3 and 4 are concerned with the production of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-73-C-807) (formerly NIH-72-C-730)

This procurement contract is designed to furnish yearly 104,000 six-week-old CD2F_1 (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonads for Drug Research and Development contract studies. The breeding animals, which originate from the genetic center at this site, are from the isolator system and are free from parasites and pathologic organisms.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-73-C-839) (formerly NIH-72-C-377)

This contract furnishes approximately 364,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

CHEMICAL ABSTRACTS SERVICE (PH43-NCI-C-65-1021)

The Chemical Abstracts Service (CAS) is operating an automated NCI chemical information system similar to the CAS Registry System. The data bank contains information on about 190,000 unique compounds submitted to Drug Research and Development for screening and on other selected compounds of interest. Included is such information as the chemical structure representation, corresponding substructure search fragments, systematic nomenclature and CAS Registry Numbers for NCI compounds. The magnetic tape files are used to determine which newly-offered compounds duplicate those already in the screen, to carry out substructure searches for analogs of compounds of interest, and

CHEMICAL ABSTRACTS SERVICE (PH43-NCI-C-65-1021) Continued

to prepare indexes of name and molecular formula cross-referenced to NSC number. Full structure searches are made of large offerings from other collections to identify nonduplicates and thereby facilitate the acquisition of new materials for screening. Some consultation in nomenclature and syste analysis is also provided.

CHICAGO, UNIVERSITY OF (NIH-NCI-C-72-3702)

This contract is intended to support studies to isolate and compare the dihydrofolate reductase from normal and malignant tissues, primarily solid tumors, in order to determine any detectable molecular differences in these enzymes. The study also utilizes in vitro testing to help design inhibitors of human and subhuman dihydrofolate reductase. These are aimed at understanding the mechanism of resistance developed in human malignancy to antifols.

CLEVELAND CLINIC (NO1-CM-33704)

This contract provides support for a protocol study in which patients with severe psoriasis will be randomly allocated to therapy with either methotrexate (MTX) on two different schedules or a control drug. Evaluation of hepatic function will be performed at regular intervals and data will be collected to determine whether hepatotoxicity in psoriasis is due to MTX or is coincidental.

COLLABORATIVE RESEARCH, INC. (PH43-NCI-C-66-506)

The major objective of this contract is the production of 5-methyl-tetrahydrohomofolic acid (NSC-139490) in large quantities. This derivative of tetrahydrohomofolic acid (NSC-89473) has improved stability properties over the parent compound, and is needed in significant quantities for preclinical toxicologic and pharmacologic evaluation. Development work on the large-sca preparation of this substance has also been done under this contract. In addition, the synthesis of some additional analogs and derivatives of homofolic acid (NSC-79249) and cytoxan has been undertaken.

COLLABORATIVE RESEARCH, INC. (NIH-NCI-C-71-2326)

The objective of this contract is the design and synthesis of oligo and polynucleotides as potential inhibitors of RNA-dependent DNA polymerase and othe DNA polymerases. It is anticipated that the synthetic nucleotides may act a templates and primers for different enzymes and may inhibit viral and leukemi DNA polymerases specifically without interfering with the normal nucleic aci metabolism of the host cells.

COLORADO STATE UNIVERSITY (NIH-NCI-C-71-2192)

The objective of this research-type contract is to investigate the pathogenes of Adriamycin/Daunomycin-induced cardiotoxicity utilizing clinical, morphological and biochemical parameters. The rabbit appears to be the most

COLORADO STATE UNIVERSITY (NIH-NCI-C-71-2192) Continued

promising species for assessing the cardiotoxicity of this class of anticancer agents. Studies have been designed to establish optimal dose and time schedules to provide the highest incidence and maximum consistency of cardiotoxicity. Adriamycin analogues are being screened for cardiotoxicity, using the rabbit, with the hope of finding one with a maximum therapeutic effect and minimal cardiotoxicity.

COLORADO, UNIVERSITY OF (NIH-NCI-C-71-2186)

Tumor tissues have consistently been shown to contain increased amounts of the enzymes which methylate pre-formed transfer RNA, the tRNA methylases. The Contractor has found naturally occurring inhibitors of the tRNA methylases in mature organ tissue, which are either lacking or inactive in malignant tissues. The purposes of this contract, then, are threefold, as follows:

1. To study the distribution and alterations of these inhibitors in tissues undergoing malignant transformation.

2. To further define the exact biochemical role the inhibitors have in the regulation of cellular differentiation and growth.

3. To determine if these inhibitors may be useful therapeutic agents for the treatment of tumors.

CONNECTICUT, UNIVERSITY OF (NIH-NCI-C-71-2181)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy, including chemotherapy, in accordance with protocols of the BTSG so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

CONTROL DATA CORPORATION (PH43-NCI-C-67-1356)

The purpose of this contract is to provide data processing and computer support which enables simple, rapid, and on-demand retrieval of clinical information related to the patient data base provided by the participating group of contractors, the BTSG who enter patients on study according to specified protocols.

DELAWARE, UNIVERSITY OF (NIH-NCI-C-69-2115)

This contract has been concerned with biochemical studies on bacterial asparaginases and glutaminases. Principal contract objective for the current year is to further purify Aerobacter glutaminase and to develop methods for its large-scale production for toxicological evaluation.

DOW CHEMICAL COMPANY (NIH-NCI-C-71-2165)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds

DOW CHEMICAL COMPANY (NIH-NCI-C-71-2165) Continued

prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram rang requiring pilot plant facilities.

DOW CHEMICAL COMPANY (NIH-NCI-C-71-2313)

The objective of this contract is the synthesis of new and novel nitrogen heterocyclic compounds as potential antitumor agents. Specifically, the Contractor is preparing compounds containing the pyrazolopyrimidine, imidaz pyrazines, pyrazolotriazine, pyrrolopyrazine and pyrazolopyrazine ring systems. These materials are postulated to be antimetabolites. All materials prepared, including intermediates, are submitted for antitumor testing.

DOW CHEMICAL COMPANY (NIH-NCI-C-72-3712)

This service-type contract is concerned primarily with reproductive studies of three antineoplastic agents in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January, 1966. These studies are divided into three phases: (1) Phase I, general reproductive performance and fertility utilizing mice or rats of both sexes; (2) Phase II, teratology studies using mice and/or rats and rabbits; and (3) Phase III, perinatal studies employing mice and/or rats.

DUKE UNIVERSITY (NIH-NCI-C-70-2030)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimenta therapy, including chemotherapy, in accordance with protocols of the BTSG so as to permit thorough analysis of comparative clinical course and surviv for experimental and control groups randomly selected.

DUKE UNIVERSITY MEDICAL CENTER (NIH-NCI-C-72-2044)

The major objectives of this contract are (a) the development of measures of sensitivity and quantity in radioautography and (b) the improvement of sensitivity and resolution of the radioautographic process.

FASEB (NIH-NCI-C-72-428)

This contract was initiated to support an NCI-sponsored symposium entitled "Fundamental Approach to Cancer Chemotherapy" which was presented at the 5t International Congress of Pharmacology, July 23-28, 1972, in San Francisco, California. The contract terminated December, 1972.

FLOW RESEARCH ANIMALS, INC. (NIH-73-C-838) (formerly NIH-72-C-378)

This contract furnishes approximately 312,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

GEORGE WASHINGTON UNIVERSITY (NIH-NCI-C-71-2206)

The objectives of this contract are to continue studies of blood flow and kinetics of drug uptake in various types of solid tumors. The canine spontaneous lymphosarcoma is used as a model for a well perfused tumor. Thermal dilution techniques and drugs such as methotrexate, guanazole, gallium and vincristine are used to characterize blood flow and membrane permeabilities to determine their relative importance in drug delivery.

GEORGETOWN UNIVERSITY (NIH-NCI-C-71-2120)

The Eastern Cooperative Oncology Group membership now consists of 33 cooperative institutions plus subsidiary hospitals and 350 members. This operations office provides executive and administrative support for activities of the Group. On request from investigators, provides randomizations and Group correspondence; obtains data from the investigators and maintains liaison with appropriate members of the staff of the NCI.

GEORGETOWN UNIVERSITY (NIH-NCI-C-71-2123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of information on the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGIA INSTITUTE OF TECHNOLOGY (PH43-NCI-C-68-1288)

The objective of the contract had been the total synthesis of the alkaloid, Camptothecin (NSC-94600), and the preparation of analogs and derivatives of it. The approach had been to devise a practical synthesis which was potentially suitable for scale-up from commercially available starting materials. Due to the reduced clinical interest in this compound, this contract terminated December, 1972.

GEORGIA, UNIVERSITY OF (NIH-NCI-C-71-2311)

The goal of this contract is the preparation of novel nitrogen heterocyclic compounds for evaluation as potential antitumor agents. The classes currently being emphasized are ethynyl oxazolidones and heteroaromatic azepines. Because of the structural similarities of these materials to those of compounds with known CNS activity, compounds prepared under this contract will be screened in intracerebral as well as intraperitoneal test systems.

HARLAN INDUSTRIES, INC. (NIH-NCI-C-72-3218)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies.

HARLAN INDUSTRIES, INC. (NIH-73-C-844) (formerly NIH-72-C-724)

This contract furnishes approximately 104,000 six-week old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HARLAN INDUSTRIES, INC. (NIH-73-C-845) (formerly NIH-72-C-419)

This contract furnishes approximately 364,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

HAWAII, UNIVERSITY OF (PH43-NCI-C-67-748)

This contract provided originally for the collection of a minimum of 500 plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the recollection of larger samples of those that are reproducibly active in the DR&D tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. Since priority was given to recollections, the general collections were reduced to a very small number. During the current year, the contract was increased to provide for 1000 general collections per year, this number to be reduced according to the number of large recollections made.

HAZLETON LABORATORIES, INC. (NIH-NCI-C-72-3701)

Mixed leukocyte cultures are an important determinant of the degree of compatibility of two individuals relative to transplantation antigens. The first objective of this contract is the selection of MLC compatible donor recipient pairs for use in an active canine and human marrow grafting program. A second objective is the continued improvement of the MLC method for use with smaller quantities of patient blood and greater reproducibility. To date 20 human experiments representing 59 blood samples and approximately three months of parallel effort in the laboratory of the Contractor and the Project Officer, have been performed in order to insure that methods in both laboratories are comparable.

HAZLETON LABORATORIES, INC. (NIH-NCI-C-72-3704)

This contract provides *in vivo* screening of new test materials, both natural products and synthetic, and the subsequent testing of active materials. The current testing level is at a rate of approximately 50,000 tests per year. The P388 leukemia is routinely used for the initial testing of natural products. Those demonstrating efficacy are also tested in L1210. The L1210 leukemia is routinely used for all new synthetic materials. Limited testing is conducted against the intracerebrally implanted ependymoblastoma, with Walker 256 Carcinosarcoma used only for the testing of synthetic analogues of materials which were active in this system. Treatment schedule studies as well as research and development work is conducted as requested by the Drug Evaluation Branch. The DR&D protocols are followed for all testing.

HAZLETON LABORATORIES, INC. (NCI-E-73-3212)

This contract provides for the supply of dogs for leukocyte transfusion and transplantation experiments presently conducted on the NIH reservation. This Contractor also supplies animals with spontaneous neoplasms in order to evaluate the immunotherapeutic effectiveness of bone marrow transplantation and immunotherapeutic manipulation following chemotherapy regimens. (Funded in part by Division of Cancer Treatment)

HAZLETON LABORATORIES, INC. (NIH-NCI-C-73-3705)

This contract provides for microbiological services in support of the NCI, Division of Cancer Treatment, clinical investigations at the Baltimore Cancer Research Center, and for special testing pertinent to this program. The services have also included microbiological surveillance and environmental studies in support of the Laminar Air Flow Room protocols of the National Cancer Institute, NIH, Bethesda, Maryland. Preliminary results of the studies are teletyped to the BCRC and NCI daily and used as indicated in research microbiological management of patients. The program employs a dataphone system for presumptive reporting and computer techniques for the retrieval of information as required by clinicians and research investigators.

HAZLETON LABORATORIES, INC. (NO1-CM-33708)

The major objectives of this contract are: (a) to obtain model tumor systems in primates induced by chemicals or viruses so that potential antitumor agents can be evaluated in these systems before undergoing clinical trial; (b) to make available normal and tumor-bearing primates as well as those with preneoplastic changes for studies of biological markers as well as chemotherapeutic and pharmacological studies; (c) to evaluate the long-term effects of antineoplastic agents currently used for long-term remissions and in treating diffuse collogen disorders; and (d) to obtain comparative data on the response of non-human primates to known rodent carcinogens and to materials suspected to be carcinogenic in man. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

HAZLETON RESEARCH ANIMALS, INC. (NIH-72-C-410)

This contract provided 150 beagle hounds for toxicologic assays. These animals were raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals were supplied by the Contractor. This contract terminated January, 1973.

HAZLETON RESEARCH ANIMALS, INC. (NIH-73-C-9)

This contract provides 180 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HAZLETON RESEARCH ANIMALS, INC. (NIH-73-C-879)

This contract provides 360 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the

HAZLETON RESEARCH ANIMALS, INC. (NIH-73-C-879) Continued

specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HEBREW UNIVERSITY (HADASSAH MEDICAL SCHOOL) (NIH-NCI-C-71-2127)

This research contract is concerned with the effects of an extract (MER - methanol extractable residue) obtained from attenuated tubercle bacilli (BCG) in stimulating the immune mechanism in animals and man with particular emphasis on antitumor activity. To date it has been found that MER can bestow marked protection of C57B1/6 mice against the leukemogenic action of the Radiation Leukemia Virus (RLV). Dosage schedule studies have shown that a requirement for MER efficacy is that first administration take place when tumor cells are already present in the host, i.e., 30 or more days after irradiation. Further studies are underway.

HERNER INFORMATION SERVICES, INC. (NIH-NCI-C-72-3244)

This contract provides for the preparation, printing and distribution of Cancer Chemotherapy Abstracts (Volumes 13 and 14, for 1972 and 1973). The price of the annual subscriptions pays the cost of photocomposition, printing and mailing by the Contractor. This current awareness service, published monthly, supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy.

HORTON LABORATORIES, INC. (NIH-73-C-837) (formerly NIH-72-C-361)

This contract furnishes approximately 208,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

3I COMPANY (NIH-71-2306)

This contract provided for the preparation of Volume 12 for 1971 of Cancer Chemotherapy Abstracts, a current awareness service which supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy. The volume consisted of six bimonthly issues and an annual cumulative index. This contract terminated September, 1972.

IIT RESEARCH INSTITUTE (PH43-NCI-C-67-1141)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two/three potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

IIT RESEARCH INSTITUTE (PH43-NCI-C-68-1021)

The major portion of this contract provides for the performance of *in vivo* testing of new synthetic materials and natural products, and also for animal health and utilization studies assigned specifically for problems that arise in various screening laboratories and animal supply contractors. Screening is being conducted according to Drug Research and Development protocol at a level of approximately 50,000 tests per year. The lymphoid leukemia L1210 in the mouse host is utilized for all synthetics. New natural products are being tested in the P388 leukemia in the mouse host. Statistical analysis and characterization studies of the first generation AKR mouse leukemia test system are continuing. Studies in this system are designed to (a) determine the correlation with the spontaneous AKR test system, (b) pick candidates for combination studies, and (c) determine the effectiveness of both early and delayed treatment in this system.

As a second function, this laboratory also maintains a routine *Salmonella spp.* and *Pseudomonas spp.* diagnostic laboratory and provides general animal disease diagnostic services as requested. These studies include virology, bacteriology, pathology and histopathology. In addition, it includes base-line studies to define the microbiological flora and virus contact status of various species of interest to the program. This portion of the contract provides the quality controls necessary for the maintenance of the quality of the animals utilized in the DR&D program.

Methodology or research and development is carried out only at the request of the Project Officer and during this year has been devoted to the development of new *in vivo* screening tumor test systems and answering specific questions that arose in the *in vivo* screening and animal health and utilization programs.

ILLINOIS, UNIVERSITY OF (NIH-NCI-C-72-2078)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant materials used in this work will be obtained, for the most part, through the U. S. Department of Agriculture.

ILLINOIS, UNIVERSITY OF (NIH-NCI-C-72-3208)

The principal objectives of this contract are as follows: (1) to provide the Division of Cancer Treatment, NCI with samples (not previously available) of a number of Ansamycin-type antibiotics for biological and related testing. This group includes the rifamycins, streptovaricins, tolypomycins and geldanamycin, at least some of which are highly potent RNA-dependent DNA polymerase inhibitors and also have antiviral activity. Thus, detailed study of as many members of this group as possible is considered imperative; (2) to isolate several presently unidentified streptovaricin components in quantity sufficient for characterization and biological evaluation; and (d) to study the metabolic fate of ansamycin compounds by isolating and identifying products formed after *in vivo* administration, from samples collected by investigators specified by NCI.

INDIAN CANCER RESEARCH CENTRE (PL-480-Agreement No. NIH-01-010-1)

This PL-480 agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing in vivo of these extracts as well as synthetic materials synthesized in India. For this purpose, an in vivo screening laboratory is maintained in Bombay, India together with an animal breeding colony to supply the necessary host animals. Currently, the L1210 lymphocytic leukemia and the P388 lymphoid leukemia are utilized with testing conducted as per current Drug Research and Development protocols. The L1210 tumor system is employed for the testing of synthetic materials with the P388 tumor system employed for the testing of natural products. Those active in this system are tested in the L1210 tumor system.

INDIANA UNIVERSITY FOUNDATION (NIH-NCI-C-70-2028)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy, including chemotherapy, in accordance with protocols of the BTSG so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

INSTITUT JULES BORDET (NIH-NCI-C-72-2053)

The objective of this contract is the maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and U. S. investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy.

INSTITUTE OF CANCER RESEARCH, ROYAL CANCER HOSPITAL (NIH-NCI-C-72-3717)

The objectives of this contract are to study the effects of chemotherapeutic agents in experimental solid tumor systems in relation to their proliferative state and their vascular supply, and compare the effects of these agents on the vascular system. To date, the effects of BCNU in Lewis Lung tumor has been studied in relation to tumor age; these findings are being correlated with kinetic parameters obtained by radioautographic methods. Comparisons are being made between chemotherapeutic responses under normal and hypoxic conditions in B16 melanoma, using cytoxan and BCNU. Studies are also underway to quantitate the effects of drugs on hematopoietic precursors. So far, it has been demonstrated that the nitrosoureas exhibit no proliferation dependence effects on normal mouse bone marrow.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION (NIH-NCI-C-71-2264)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of one potentially useful antineoplastic agent in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION (NIH-NCI-C-71-2264)Continued

The toxicity is characterized as to predictability, reversibility, and schedule dependency of the toxicity. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

INSTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" (NIH-NCI-C-72-3242)

The principal objective of this contract is to investigate drug distribution and drug interactions in rodents bearing transplantable tumors. Studies have been carried out to determine blood and tumor levels of methylnitrosourea, methotrexate and 6-mercaptopurine in mice bearing Sarcoma 180. The previous treatment of animals with cyclophosphamide on the above drug levels has also been studied. The relationship between antitumor activity and tumor concentrations of these above drugs is being carried out in mice bearing Erhlich carcinoma transplanted in different sites.

JACKSON LABORATORY (NIH-71-1004)

This service contract provided for the procurement of approximately 112,600 C57BL/6 and 31,400 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplied 124,800 AKR inbred mice for compound evaluation studies. Breeding animals were supplied by the Contractor. This contract terminated July, 1972.

JACKSON LABORATORY (NIH-72-C-1171)

This service contract provides for the procurement of approximately 78,000 C57BL/6 and 31,200 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplies 78,000 AKR inbred mice for compound evaluation studies. Breeding animals are supplied by the Contractor.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NIH-NCI-C-72-2054)

The objective of this contract is the maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and U.S. investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy.

JEFFERSON MEDICAL COLLEGE (NIH-NCI-C-70-2136)

The purpose of this contract is to conduct clinical pharmacologic studies of new antineoplastic drugs entering phase one trial at the University Hospital. Basic investigation includes development of analytical methods, determination of plasma disappearance, and studies of drug metabolism and excretion, and other pertinent parameters. Thus far such coordinated trials have been

JEFFERSON MEDICAL COLLEGE (NIH-NCI-C-70-2136) Continued

conducted with dibromodulcitol, streptozotocin, and 5-azacytidine, and a new series of studies with the Yoshi compound (NSC-102627) are planned in the coming year.

KANSAS, UNIVERSITY OF (NIH-NCI-C-71-2010)

This Contractor maintains a genetic production center. Its most important function is to secure the maintenance of the strains of rodents that are of interest in Drug Research and Development program investigations. Its spectrum includes six strains: C57BL/6, DBA/2, AKR, BALB/c, C3H/He, and A/He. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies. Hybrids for special studies are produced as required for specific studies.

KANSAS, UNIVERSITY OF (NIH-NCI-C-71-2184)

The principal objective of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. Methotrexate and 5-fluorouracil are currently under study.

KANSAS, UNIVERSITY OF (NIH-NCI-C-72-3217)

This contract provides research capabilities in the area of formulation design and development. The research involves the development of intravenous dosage forms of new drug substances having formulation problems. A second objective of this research contract is to develop new and unique delivery systems for poorly manageable drugs. The drugs to be researched are assigned by the NCI staff through the Project Officer.

KENTUCKY, UNIVERSITY OF (PH43-NCI-C-67-1173)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy, including chemotherapy, in accordance with protocols of the BTSG so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

LABORATORY SUPPLY COMPANY (NIH-NCI-C-70-2041)

This Contractor maintains a genetic production colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

LABORATORY SUPPLY COMPANY (NIH-73-C-835) (formerly NIH-72-C-733)

This contract furnishes approximately 104,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LABORATORY SUPPLY COMPANY, INC. (NIH-73-C-840) (formerly NIH-72-C-360)

This contract furnishes approximately 312,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LEO GOODWIN INSTITUTE FOR CANCER FOR CANCER RESEARCH (NO1-CM-33729)
(formerly Germfree Life Research Center - NIH-NCI-C-72-3204)

This primary genetic center has as its objectives the development of germfree foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germfree isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTLE, ARTHUR D., INC. (NIH-NCI-C-72-3257)

This project encompasses the review and analysis of the present Drug Research and Development Biological Data Processing System. The objectives are to provide recommendations for improving and expanding the present system in order to better evaluate and summarize primary screening data for the selection of compounds for secondary screening, to develop methods for the evaluation and reporting of compounds tested in the secondary screen, and for incorporating the secondary screening data into the present system. A primary goal is to provide suppliers with reports in a form of most use to them; this involves periodic summary reports of all compounds submitted during a year; the suppliers' laboratory number, the NSC number assigned, and systematic chemical names will be listed along with a brief English language statement indicating whether the compound showed activity in our present screen according to current criteria. The organization of the file is also being studied with a view to increasing flexibility and effecting economies.

LITTLE, ARTHUR D., INC. (NIH-NCI-C-72-3711)

This contract provides broad biomedical engineering resources which are responsive to the support of individual research projects within the Division of Cancer Treatment. A flexible mechanism of support is being tested to define and implement engineering projects in close collaboration with the Division of Cancer Treatment staff in active areas such as: automated microspectometry, cell kinetics modeling, the automation of grain counting and bone marrow colony assays, and studies of the protective laminar flow environment for supportive care of leukemic patients. A "core group" of senior engineers continually consults and interacts with the NCI staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under staff direction.

LITTLE, ARTHUR D., INC. (NO1-CM-33727)

This is a broad-based major research and development program which is directly aligned to the overall mission of the NCI Division of Cancer Treatment Program. The task objectives are divided into the following major components:

This Screening and Experimental Therapeutics and Tumor Biology effort provides for routine primary screening in in vivo systems of synthetic and natural products, the detailed evaluation of active drugs, schedule dependency and combination chemotherapy studies and the development of new and improved methods for in vivo screening and detailed drug evaluation. The Cell Kinetics studies involve the measurement of generation times, doubling times and mitotic indices of solid and ascitic tumors via various techniques, investigation of effects of selected drugs on intravenous-inoculated tumor cells in liver, spleen and bone marrow to establish whether there are preferential sites of in vivo cytotoxicity, investigations of hematological recovery in the bone marrow of normal drug-treated mice for the purpose of determining optimal therapy regimens, and other methodological studies which may be considered appropriate.

The Applied Mathematical and Computer Oriented services provide for the analysis of studies from all appropriate contract laboratories related to the development and finalization of new protocols for screening. The principal objective is to advise Drug Research and Development regarding experimental design, parameters and criteria for activity and data analysis.

The Cell Culture and Related Methodology work consists of routine in vitro screening, bioassay of natural product fractions, detailed drug evaluation in vitro, and the development of improved assays for natural product bioassay.

The Neuropharmacology studies are aimed at approaching various toxicological and pharmacological problems relating to the treatment of neoplastic diseases of the central nervous system. Improved techniques are being sought for the administration of agents used to control neoplastic disease in the CNS.

The Central Nervous System solid tumor studies are concerned with the development and evaluation of transplantable gliomas in the CNS of beagle dogs, together with collaborative studies with clinical and pharmacological groups of the NCI to examine the growth kinetics and the behavior of transplantable gliomas to selected agents.

The Preclinical Toxicology portion of this contract is concerned with the preclinical toxicologic evaluation of three potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

LITTLE, ARTHUR D., INC. (NO1-CM-33727) Continued

The Physiologic Disposition and Biochemistry effort provides for sophisticated exploratory research on the mode of action and physiological disposition of active agents, and includes a cooperative project with selected clinical groups to measure the metabolic fate and physiologic disposition of drugs being employed in the management of cancer in humans.

The Cryobiology and Tumor Distribution activities provide for the maintenance of a frozen tumor and cell bank, distribution of standard tumor lines to screening laboratories and other research institutions, and methodological studies on the frozen, stored tumor lines.

The Electron Microscopy and Radioautography program provides such services in support of the pharmacological, toxicological, microbiological and solid tumor studies.

LITTON BIONETICS, INC. (NIH-NCI-C-71-2340)

The objectives of this project are to develop new approaches for chemotherapy of acute leukemia by searching for specific inhibitors of RNA dependent DNA polymerase and of other leukemic (human) DNA polymerases to expand basic research efforts in tumor cell biochemistry in general in order to achieve a better understanding of the neoplastic process and its prevention and treatment.

LITTON BIONETICS, INC. (NIH-NCI-C-72-2062)

The main objective of this contract is the preparation, growth and characterization of defined tissue culture cell lines for use by Project Officers. Cell cloning, cell genetics, defining immunological cell markers and labeling cells with radioisotopic precursors for the isolation of metabolic products are also performed.

LITTON BIONETICS, INC. (NIH-NCI-C-72-3294)

The purpose of this contract is to provide for the management, operation and maintenance of the Frederick Cancer Research Center, involving a broad spectrum of cancer research activity.

Projects 11 and 12 of the contract, which are specifically related to the NCI Division of Cancer Treatment program, provide for the production of inbred and hybrids of inbred mice, inbred rats, guinea pigs and rabbits. These laboratory animals are supplied primarily for investigations performed at the Frederick Cancer Research Center, and as available to NCI laboratories in Bethesda and NCI contract-supported laboratories located in various geographic areas of the contiguous United States. Breeding animals originate from the barrier-type colonies maintained by the Veterinary Resources Branch, DRS, OD, NIH. Construction activities commenced during this first year of activity are programmed for the conversion of all buildings to barrier-type status. The animal farm complex includes a quality control laboratory in order to ensure that the animals will be free from pathogenic microorganisms.

LITTON BIONETICS, INC. (NO1-CM-33741)

This contract provides a source for the screening of up to 1,000 compounds per year, as selected by NCI, for inhibitory activity for selected mammalian cell and viral enzymes in test tube systems and for toxicity or specific inhibition of oncogenic viral replication and viral transforming capacity in cell culture systems. Enzymes employed in the test tube inhibition studies include the viral polymerase of Avian Myeloblastosis virus as well as the DNA polymerases and RNA polymerase II isolated and purified from bovine tissues, and other systems as designated. The screening operation includes a preliminary tissue culture and antienzymatic screen for all compounds and a secondary tissue culture and antienzymatic screen for compounds demonstrating significant activity in the primary screen.

MAKERERE UNIVERSITY COLLEGE COUNCIL (NIH-NCI-C-67-1343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to provide facilities for direct collaborative studies to the members of the NCI, including the Medical Oncology Area, Laboratories of Pharmacology, Immunology, Pathology and Etiology. Research is continuing as previously but under the direction of Sir Ian W. J. McAdam and Dr. Sebastian Kyalwazi.

"MARIO NEGRI" INSTITUTE FOR PHARMACOLOGICAL RESEARCH (NO1-CM-33720)

The main purpose of this project is to establish a regional European contract as an extension of the DCT compound acquisition and screening program. The Contractor will collect 500 to 1,000 materials in Southern European countries and perform primary screening at a rate of approximately 7,000 L1210-equivalency tests per year.

MARSHALL RESEARCH ANIMALS, INC. (NIH-73-C-889) (formerly NIH-72-C-364)

This contract provides 360 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MASON RESEARCH INSTITUTE (NIH-NCI-C-70-2055)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of nine potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

MASON RESEARCH INSTITUTE (NIH-NCI-C-71-2259)

This service contract is for the in vivo testing of synthetic and natural products which are submitted to Drug Research and Development. Primarily the L1210 tumor system is utilized for the testing of synthetic materials with the B16 Melanoma used for selected materials. New natural products are tested initially in the P388 leukemia with those demonstrating efficacy being tested in the L1210 leukemia. Testing is proceeding at a level of approximately 37,000 L1210 equivalent tests per year. Schedule dependency studies to determine the optimal dose, schedule and route of administration as well as combination studies are conducted as directed by DR&D staff. A modest portion of the effort is devoted to the genetic characterization of inbred rats used for program studies.

MAYO FOUNDATION (NIH-NCI-C-70-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment Program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at NCI. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MAYO FOUNDATION (NIH-NCI-C-71-2185)

This contract is designed to provide information on the effect of chemotherapy on the growth and size of breast cancer lesions and toxicity. Selection of drugs and protocol is by mutual agreement between the Project Officer and the Mayo Foundation. During the initial six months of the contract, the Mayo Foundation involved 49 patients in chemotherapeutic trials.

MAYO FOUNDATION (NO1-CM-33735)

This is one of three Medical Oncology contracts effective in January 1973 for the conduct of eradicative clinical trials of patients with bronchogenic carcinoma. Comprehensive vertical treatment plans will be adapted to the characteristics of the major types of lung cancer, comparing best available treatment with promising new approaches to therapy.

MEDICAL COLLEGE OF VIRGINIA (NIH-NCI-C-69-2266)

This contract is concerned with testing bacterial endotoxins of Escherichia coli and Salmonella, and exotoxins of Streptococcus and Staphylococcus species for possible synergistic toxicity with selected cancer chemotherapeutic agents.

MEMORIAL HOSPITAL (NIH-NCI-C-72-2073)

This contract is designed to conduct Phase II studies of anticancer agents in patients with metastatic adenocarcinoma of the breast. This is being performed

MEMORIAL HOSPITAL (NIH-NCI-C-72-2073) Continued

as a cooperative venture between Memorial Hospital and the NCI to test selected agents, either developed by the NCI or of interest to them. The current study is a comparison of adriamycin and CCNU but since this has been in effect for too short a time to permit definitive evaluations of a large number of patients, conclusions regarding relative effectiveness of the two agents cannot yet be drawn. It is planned to continue the present comparative study until 50 patients have been evaluated.

MERCK AND COMPANY (NIH-NCI-C-72-2002)

This contract provides for the resynthesis of compounds in the amounts needed for clinical trial or for necessary preclinical evaluations. About 25% of the budget is devoted to clinical preparations.

This is the largest and oldest of the Drug Research and Development preparative laboratories. Because of their extensive and diverse experience, the Contractor is able to prepare compounds of many different and difficult types. These are materials for which no commercial source is available or which cannot be prepared in the required quantities by the original supplier.

MIAMI, UNIVERSITY OF (NIH-NCI-C-70-2225)

This contract provides for the in vitro presumptive screening of synthetics and natural products and the follow-up tests of natural products having demonstrated correlative activity in the Drug Research and Development in vivo screening program, as a tool for fractionation. Procedures are those described by Drug Research and Development protocols and methodology is carried out only at the request of the Project Officer. Approximately 15,000 tests at three to five dose levels per test are performed yearly.

MICHIGAN DEPARTMENT OF HEALTH (NIH-NCI-C-73-3702)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program serviced by this assay contract is financed by the Michigan Department of Health.

MICHIGAN, UNIVERSITY OF (NIH-NCI-C-69-2004)

This contract is for the chemical synthesis of a number of new nitrogen heterocyclic compounds containing complex ring systems. The compounds are for primary screening and are unique structures which have been little explored and/or are heretofore unknown. All materials are fully characterized, of high purity and in sufficient quantity for adequate evaluation.

MICHIGAN, UNIVERSITY OF (NIH-NCI-C-72-3714)

The objective of this contract is to help delineate the role of RNA in DNA replication in human cells. RNA molecules may serve as initiator molecules (primers) in DNA directed (templated) DNA synthesis in phage-bacterial systems and probably in mammalian cells as well. RNA also may direct (template for) DNA synthesis (so called reverse transcriptase reactions); the latter has now been found in RNA tumor viruses, human acute leukemic cells, chick embryo, and in some established tissue culture tumor cell lines. Both types of reactions are potentially relevant to chemotherapy, the former in DNA replication (a target for some useful antitumor agents) and the latter in replication of RNA tumor viruses. Progress in the last year has been made in the identification and preliminary characterization of an RNA primer human lymphoblast cell line.

MICROBIOLOGICAL ASSOCIATES, INC. (NIH-NCI-C-69-2177)

The Contractor performs routine serologic assays for the determination of alloimmunization on NCI patients and potential platelet and leukocyte donors. This Contractor serves as a serum repository for serialized serum specimens from all patients in the Pediatric Oncology Branch program.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-33728)

This particular program is the product of the realignment and gradual consolidation, over a number of years, of individual contracts with this Company (both service and research-oriented) which now forms a single broad-based coordinated mechanism for the detailed investigation of problems which arise in the chemotherapy of malignant disease. The total program is closely integrated scientifically with NCI operations, and the activities are carried out under the daily guidance of professional staff of Drug Research and Development and the Experimental Therapeutics areas of the NCI Division of Cancer Treatment Program. Projects selected for study are those which are determined to be highly pertinent to the needs of the preclinical and clinical programs of the NCI. The contract is divided into eight basic divisions, each of which is closely monitored by an Assistant Project Officer.

Chemotherapy Research - Activities in this division involve exploratory research in biological and biochemical areas of chemotherapy, and include studies on cell kinetics, the search for and evaluation of new screening methods, studies related to remission induction and maintenance, etc.

Drug Evaluation - The principal current emphasis is on the rapid screening of synthesis contractor analogs in in vivo assays designed specifically on the basis of the characteristics of the parent active agent. Considerable emphasis is placed on the evaluation of new and improved in vivo assays for primary screening. In addition, this division conducts a considerable portion of DR&D studies related to the detailed evaluation of active drugs of clinical interest.

Immunology - The principal objectives of this project are the development of immunotherapy models, basic studies on the immunology of viral oncogenesis, and detailed drug evaluation using the spontaneous AKR lymphoma in vivo and Moloney

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM-33728) Continued

virus induced tumors in vivo and in vitro. Emphasis is placed on evaluating drugs as antiviral agents in vitro, antiviral agents in vivo (prophylaxis) and as antitumor agents against primary virus-induced tumors.

Toxicology - The toxicology portion of this contract is directed toward: (1) the study of the effect of oncolytic agents on the cardiovascular system; (2) the mechanism of cardiotoxicity; (3) development of methods for the prevention or modification of the cardiotoxic effects; and (4) other pharmacodynamic studies of antineoplastic agents.

Drug Metabolism - This group functions as a source for the study of the physiological distribution and metabolism of antitumor drugs, with special attention to their relationship to therapeutic effectiveness and toxicity.

Organic Chemistry - This portion of the contract is directed to the synthesis of new compounds of potential usefulness, the examination of chemical problems of drug development, and studies on the structure of new agents.

Drug Distribution - This division provides services to the NCI for the storage and distribution of drugs and chemicals to various research laboratories throughout the country.

Laboratory Animal Science - The function of this division is primarily the operation of a genetic center for mouse strains needed in support of the research activities of the contract. Holding facilities and animal caretaking services are a major function of this project.

MIDWEST RESEARCH INSTITUTE (NIH-NCI-C-71-2263)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency of the toxicity. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

MIDWEST RESEARCH INSTITUTE (NO1-CM-33722)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for Drug Research and Development. The Contractor determines the identity and purity of these materials, developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromotographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation.

MIDWEST RESEARCH INSTITUTE (NO1-CM-33743)

Midwest Research Institute is one of three major synthetic contractors for Drug Research and Development. The primary objective of this contract is the rapid exploitation of leads generated by the Drug Evaluation Branch and clinical groups. This Contractor has provided a large number of compounds to DR&D for screening. These compounds represent the exploration of a variety of leads. Current investigation centers around the synthesis of (1) 1,4-bis (alkoxy-substituted phenyl) butane and tropolones as potential t-RNA o-methyl-transferase inhibitors, (2) receptor site-oriented antileukemic compounds based on the N-O-O triangulation hypothesis developed by this Contractor, and (3) coralyne, bleomycin and other natural products and their analogs. The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MISSOURI, UNIVERSITY OF (NIH-NCI-C-71-2323)

The objective of this contract is to develop analytical procedures involving the sensitive GLC techniques to measure quantitatively specific and potential biologic markers in the urine and blood of cancer patients and to utilize these procedures to study patients before, during and after therapy. To date, methods have been established for assay of polyamine (putrescine, spermidine, cadaverine and spermine) and methylated nucleic acid derivatives, as well as other minor nucleic acid bases, in urine, and clinical studies have begun to determine the value of these markers for following disease status in patients. In addition, preliminary work has been initiated for the assay of several other potential markers in serum and urine.

MONSANTO RESEARCH CORPORATION (NIH-NCI-C-72-3715)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the NCI staff.

MONSANTO RESEARCH CORPORATION (NIH-NCI-C-73-3709)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities. This contract also provides for the large-scale extraction, isolation and purification of natural products required for preclinical and clinical studies.

MURPHY BREEDING LABORATORIES, INC. (NIH-73-C-868)

This procurement contract provides for the supply of 104,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the Drug Research and Development compound

MURPHY BREEDING LABORATORIES, INC. (NIH-73-C-868) Continued

evaluation program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

NATIONAL ACADEMY OF SCIENCES (PH43-NCI-C-64-44 - TASK ORDER #6)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (PH43-NCI-C-64-44 - TASK ORDER #16)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of bio-medical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts, and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL BUREAU OF STANDARDS (NIH-NCI-C-69-49)

This effort supports an NCI investigation of automatic grain counting and blood cell identification through the processing of scanned images of cells, the experimentation with computer algorithms for image analysis, grain counting and instrument controls and the provision of engineering assistance in the development and interfacing of software and hardware components of NCI laboratories conducting the primary investigations.

NEW JERSEY COLLEGE OF MEDICINE AND DENTISTRY (NIH-NCI-C-72-3211)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection of antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen

NEW JERSEY COLLEGE OF MEDICINE AND DENTISTRY (NIH-NCI-C-72-3211) Continued

and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NIH-NCI-C-70-2035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling Drug Research and Development to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NIH-NCI-C-72-2072)

This contract provides support in the evaluation of the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival using mutually agreeable protocols. Thirty patients have been entered in a protocol comparing 5-azacytidine with CCNU. The overall incidence of tumor remissions induced by both drugs has been lower than would have been expected from other standard agents such as 5-FU or from combination. It is anticipated that this study will be terminated in the near future and another one planned.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-33726)

The objective of this contract is the development of antiviral-antitumor agents from a class of ansa macrolide compounds, the streptovaricin complex. These agents were first shown by the principal investigator to be reverse transcriptase inhibitors and to interfere with viral replication. A significant finding has been their later observation of relatively selective in vivo effects, i.e, minimum cytotoxicity yet interference with viral replication. Attempts are underway to isolate the active component(s) from the crude complex.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-33734)

This contract is designed to determine the relative efficacy of current therapeutic approaches to the treatment of lung cancer; current staging techniques as a reliable index of prognosis; the significance of morphologic and histologic characteristics of lung cancer; to explore new and promising avenues of therapy for lung cancer; devise and implement therapeutic protocols that will lend to a systematic and progressive understanding of lung cancer as a disease and promote its eventual control; and to foster both national and international group collaboration, correlating all efforts and knowledge in such a manner as to avoid wasteful expenditure of time and talent that would distract from the

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NO1-CM-33734) Continued

earliest possible conquest of the disease. This is a new contract and results are not available as yet.

NEW YORK UNIVERSITY MEDICAL CENTER (PH43-NCI-C-67-1174)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy, including chemotherapy, in accordance with protocols of the Brain Tumor Study Group so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

NOTRE DAME, UNIVERSITY OF (NIH-NCI-C-72-3718)

Leukemia patients under treatment by chemotherapy are highly vulnerable to infection. Microorganisms that are part of the normal human flora may themselves cause serious infection. This contract was activated in 1972 and work being done is directed toward determining whether normal control can be effected biologically. Specifically, the question being asked is whether the normal, potentially hazardous flora can be replaced with an innocuous, readily controllable flora. If this can be achieved, it may be possible to provide chemotherapy under much less stringent conditions than required at present.

OHIO STATE UNIVERSITY (PH43-NCI-C-67-1175)

The purpose of this contract is to provide clinical data from selected patients with brain tumors in order to evaluate the efficacy of experimental therapy, including chemotherapy, in accordance with protocols of the Brain Tumor Study Group so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups randomly selected.

OHIO STATE UNIVERSITY (NO1-CM-33736)

This is one of three Medical Oncology contracts effective in January 1973 for the conduct of eradicative clinical trials of patients with bronchogenic carcinoma. Comprehensive vertical treatment plans will be adapted to the characteristics of the major types of lung cancer, comparing best available treatment with promising new approaches to therapy.

OREGON STATE UNIVERSITY (NIH-NCI-C-71-2191)

This research-type contract is investigating the mechanism of the delayed marrow toxicity of BCNU and related oncolytic nitrosoureas in several animal species. The exact timing of the toxic suppression of erythropoiesis caused by BCNU is being characterized by studying the rate of uptake of ^{59}Iron into hemoglobin of animals given graded doses of BCNU. This contract is being terminated this fiscal year.

OREGON STATE UNIVERSITY (NIH-NCI-C-72-3201)

The purpose of this contract is to acquire pharmacologic disposition information from animals so that such information is available prior to initiating clinical

OREGON STATE UNIVERSITY (NIH-NCI-C-72-3201) Continued

trials. Specifically, the three nitrosoureas, BCNU, CCNU and MeCCNU are being studied from two points of view. First, the complicated metabolic alterations these drugs undergo are being studied in detail. Second, the effects of these agents on the ability of the host hepatic microsomes to metabolize other drugs is under investigation.

PARKE, DAVIS & COMPANY (NIH-NCI-C-72-3708)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. About 50% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities. This contract also provides for the large scale extraction, isolation and purification of natural products required for preclinical and clinical studies.

PENNSYLVANIA, UNIVERSITY OF (NIH-NCI-C-71-2187)

This contract provided for the development of specifications of a detailed system design for an extended Drug Research and Development chemical information system to replace the current semiautomated system. The extended system provided improved capability to: (1) identify which newly received compounds were new to the Cancer Treatment Program, including the flagging of analogues and duplicates of compounds of interest; (2) automatically produced computer-generated shipping documents and other communications to suppliers, screeners, and in-house staff; (3) responded readily to a significant increase in samples, with rapid turnover from receipt by the Program to shipment for screening; (4) conducted substructure search with simplified inquiry procedures and structure output; (5) maintained on-line inventory control; (6) maintained improved data files, eliminated several manual files, and furnished a variety of retrieval and reporting requirements; (7) linked the chemical and biological data files; (8) maintained management and quality control of the system; (9) permitted the user to be information oriented, not computer system oriented; and (10) expanded to a fully interactive system with increased user participation This contract terminated November 1972.

PENNSYLVANIA, UNIVERSITY OF (NIH-NCI-C-71-2188)

The purpose of this contract is to determine whether there may be useful anti-cancer agents whose prime mechanism of action is selective inhibition of cellular energy production. To accomplish this, a study is being made of the correlation between *in vivo* anticancer activity and the effects on mitochondrial energy coupling reactions and electron shuttle mechanisms of ascites tumor cells *in vitro*.

PENNSYLVANIA, UNIVERSITY OF (NIH-NCI-C-73-3719)

This contract provides for implementing the design of and installing an extended chemical information system to replace the current semiautomated system of the Division of Cancer Treatment. This effort will include: (1) writing, testing

PENNSYLVANIA, UNIVERSITY OF (NIH-NCI-C-73-3719)

and documenting computer programs; (2) testing the system on a program, subsystem and total integrated basis, as well as in parallel with the existing system; (3) designing data collection, transcription and report forms; (4) converting existing and generating new data files; (5) preparing users' guides and training manuals, covering all aspects of system operation and management; (6) training operational and management staff; and (7) reviewing the performance of the new system.

The new system will provide improved capability to: (1) identify which newly received samples are new to the Division of Cancer Treatment Program, including the flagging of analogues and duplicates of compounds of interest; (2) produce computer-generated shipping documents and communications to suppliers, screeners and in-house staff; (3) conduct substructure search with simplified inquiry procedures and structure output; (4) maintain on-line inventory control; (5) maintain improved data files, eliminate several manual files and furnish a variety of retrieval and reporting requirements; and (6) maintain management, operational and quality control of the system.

PENNWALT CORPORATION (NIH-NCI-C-71-2309)

The objective of this contract is the synthesis of coordination compounds. Few transition metal coordination compounds have been adequately investigated as potential antitumor drugs. This contract will synthesize coordination compounds of platinum, rhodium, niobium and tantalum. They will also prepare cluster compounds of niobium and tantalum for antitumor evaluation. This area of synthetic investigation may be expanded to include additional classes of metal complexes if screening test results warrant.

PHARM-ECO (NIH-NCI-C-72-3707)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. About 50% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range.

PHILIPS ROXANE LABORATORIES, INC. (NIH-NCI-C-71-2139)

This service contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms prepared at Philips Roxane Laboratories are packaged, labelled and shipped to NCI for subsequent distribution to clinical investigators.

POMONA COLLEGE (NIH-NCI-C-73-3703)

This project has two major objectives. First is the attempt to derive a structure-activity correlation for anticancer drugs. This will be done using currently available data and the statistical methods of linear regression

POMONA COLLEGE (NIH-NCI-C-73-3703) Continued

analysis developed by Dr. C. Hansch of Pomona College. The second objective is the accumulation of fundamental data relating to hydrophobic bonding. These data will be used for further refinement of the structure-activity correlation as well as studies of the mechanisms of interaction of "mini-molecules" (drugs) with macromolecules (enzymes).

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PROGRAM SERVICES (TRANSFER OF FUNDS - NCI-C-64-3) (Baltimore, Maryland)

This transfer of funds permits service support by the Hospital of NCI-intramural cancer chemotherapy programs at the USPHS Hospital in Baltimore, Maryland. Programs of clinical pharmacology, clinical use of the newer drugs for the treatment of cancer, and developments in improved patient care are on-going projects. Initial clinical trials of agents developed entirely within the NCI Division of Cancer Treatment Program and fundamental related laboratory research represent significant portions of the program.

RAWLEY FARMS (NIH-72-C-380)

This contract furnished approximately 390,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals were furnished from genetic centers and/or rodent production colonies. This contract terminated March 1973.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (NIH-NCI-C-73-3715)

This contract encompasses statistical and operational support for the Working Party for Therapy of Lung Cancer and includes activities conducted by the biostatistician and by the Operations Office of a multidisciplinary, cooperative study group. Additional responsibilities include central randomization and stratification of patients who enter studies and preparation and monitoring of monthly fiscal reports for the members of the Working Party, progress reports by membership institutions every four months, and annual reports. The contract was activated in September of 1972.

RESEARCH TRIANGLE INSTITUTE (NIH-NCI-C-69-2019)

The purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compounds. Plant material used for this work is obtained, for the most part, through the U. S. Department of Agriculture. The Contractor has many plants which are, or have been in some stage of fractionation. Several active agents have been isolated and are currently being subjected to additional evaluation. A KB cell culture facility has been added to expedite the fractionation work.

RHODE ISLAND, UNIVERSITY OF (NIH-NCI-C-71-2312)

This contract provides for the synthesis of nitrogen heterocyclic compounds with potential antitumor properties. The compounds being synthesized are analogs and derivatives of the imidazolopyrazine and isoxazolopyrazine ring

RHODE ISLAND, UNIVERSITY OF (NIH-NCI-C-71-2312) Continued

systems. Both systems have structural similarity to purines and have the possibility of interfering with normal purine metabolism.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NIH-NCI-C-72-2042)

The objective of this contract is to prepare and test analogs of the coenzyme form of vitamin B_{12}. These are to consist of alterations in both base and/or sugar of the adenosine portion of the molecule. Synthesis will be either enzymatic or chemical. After establishing identity of the agents, they are to be tested first for cell penetration and anti-B_{12} activity in vitro. Potentially useful agents are then to be made in larger quantities for in vivo testing for anticancer activity.

SHEEHAN INSTITUTE FOR RESEARCH (NIH-NCI-C-72-3719)

The objective of this contract is the synthesis of new compounds with potential as central nervous system (CNS) antitumor agents. Specifically, compounds related to ergocoronine, which are CNS active and which may pass the blood-brain-barrier, will be synthesized and evaluated in appropriate intracerebral test systems. The compounds prepared will also be evaluated under standard screening protocols.

SIMONSEN LABORATORIES, INC. (NIH-NCI-C-71-2101)

The Contractor maintains a genetic production colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

SIMONSEN LABORATORIES, INC. (NIH-72-C-725)

This procurement contract furnished yearly 104,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for Drug Research and Development studies. Breeding animals originated from genetic centers. The mice were offered chlorinated water in order to eliminate or control the incidence of pseudomonads. This contract terminated April 1973.

SIMONSEN LABORATORIES, INC. (NIH-73-C-816) (formerly NIH-72-C-409)

This contract furnishes approximately 364,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SIMONSEN LABORATORIES, INC. (NIH-NCI-C-72-3219)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTH SHORE ANALYTICAL AND RESEARCH LABORATORIES, INC. (PH43-NCI-C-64-932)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two/three potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced deaths. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

SOUTHERN ANIMAL FARMS (NIH-72-C-732)

This procurement contract furnishes approximately 104,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for Drug Research and Development contract studies. Breeding animals are furnished from genetic centers and/or rodent production colonies. This contract will terminate in April 1973.

SOUTHERN ANIMAL FARMS (NIH-NCI-C-72-2013)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN ANIMAL FARMS (NIH-73-C-863) (formerly NIH-72-C-379)

This contract furnishes approximately 364,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NIH-NCI-C-72-3241)

The purpose of this contract is to define the pharmacokinetics of two new antineoplastic agents, adriamycin and guanazole, in experimental animals and man. The Contractor has accordingly developed methods for assay of parent drug and metabolites of adriamycin, and has begun the collection of data in the rat and rabbit in order to construct a comprehensive pharmacokinetic model, which will then serve as the basis for studies in man.

SOUTHERN RESEARCH INSTITUTE (PH43-NCI-C-65-654)

The work supported by this contract represents a portion of an integrated effort to obtain basic quantitative information on the requirement of drug dosage and scheduling which will permit maximum tumor cell kill while minimizing toxicity to the host's normal cells. The major research effort has been devoted to the development of basic analytical methodology including microbiological assays for the estimation of antitumor drugs in biological materials. After the method

SOUTHERN RESEARCH INSTITUTE (PH43-NCI-C-65-654) Continued

is developed it is applied to gain quantitative information on the absorption, distribution and fate of the agents in various species. During the past year, studies have been conducted on ara-C, cyclocytidine, tetrahydrouridine combination pharmacology of 6-thioguanine and ara-C, actinomycin D, nitrosoureas isophosphamide and rifampicin.

SOUTHERN RESEARCH INSTITUTE (PH43-NCI-C-66-29)

The major objective of this contract is to develop basic biochemical knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim, studies are underway on: (1) sites of action and the metabolism of compounds with antitumor activity; (2) the biochemical basis for resistance to important antitumor agents; and (3) the comparative biochemistry of tumors and various normal cells.

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-C-70-2083)

The principal objective of this contract is to develop methodologic approaches to evaluating the toxicities of combinations of drugs in animal model systems and to apply these methods to studies of combinations for clinical trials. Studies currently underway concern (1) the toxicity of repeated courses of arabinosyl cytosine (ara-C) and 6-thioguanine in Rhesus monkeys, (2) the toxic effects of repeated doses of CCNU and cyclophosphamide in the Rhesus monkey and beagle dog, and (3) the study of the rate of host recovery in mice receiving such agents as ara-C, cyclophosphamide, MeCCNU, adriamycin and methotrexate.

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-C-71-2098)

Objectives of this comprehensive research and development contract are: (1) development of biological principles leading to discovery of more effective anticancer therapy, (2) development of improved assays for predicting clinical usefulness, (3) detailed evaluation of new drugs as potential clinical candidates, and (4) primary screening in vivo and in vitro. Objectives are realized through (a) basic studies of factors influencing growth of spontaneous and transplanted animal tumors, (b) studies of the dynamics of tumor cell proliferation and the effects of drugs thereon, and (c) quantitation of drug activity relative to factors which influence it (e.g., treatment route and schedule). Studies of drug combinations and studies of resistance and cross-resistance are conducted to uncover superior therapies leading to cures of animal tumors and in conjunction with studies of mechanisms of drug action. Mammalian cell culture research and development is aimed at defining the influence of drug concentration and duration of effect at the target cell, and the cell cycle stage specificity of active drugs. Antimicrobial testing is aimed at providing information relative to mechanisms of drug action and at identifying suitable organisms for bioassay of effective drug levels in body fluids.

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-C-71-2189)

The purpose of this contract is to study the effect of potential anticancer agents on cell membrane formation. This is being approached by determining the

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-C-71-2189) Continued

effects of these drugs on incorporation of labeled precursors into various membrane constituents such as glycoproteins, sphingolipids, phospholipids, cholesterol, etc.

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-C-73-3712)

Southern Research Institute is one of the basic synthesis contractors for Drug Research and Development. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening, therapeutic and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines, ring analogs of purines, and nucleosides; folic acid antagonists, directed toward the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and analogs of imidazoles; nucleotides; N-nitrosourea analogs; aryl and heterocyclic triazenes; metabolites of cytoxan; compounds containing very reactive chemical moieties; and analogs of mitomycin.

SPARTAN RESEARCH ANIMALS, INC. (NIH-73-C-858)

This procurement contract provides for the supply of 104,000 $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice yearly for the Drug Research and Development compound evaluation program. Breeding animals originate in genetic centers.

SPARTAN RESEARCH ANIMALS, INC. (NIH-73-C-878)

This procurement contract provides for the supply of 104,000 $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the Drug Research and Development compound evaluation program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

STANFORD RESEARCH INSTITUTE (NIH-NCI-C-71-2162)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the NCI staff.

STANFORD RESEARCH INSTITUTE (NIH-NCI-C-72-3200)

The purpose of this contract is to acquire pharmacologic disposition information from animals so that such information is available prior to initiating clinical trials. The following investigations are conducted: (1) plasma levels and urinary, biliary and fecal excretion rates of drugs and/or metabolites of compounds of interest to the chemotherapy program; (2) tissue distribution of the parent drug and metabolites after oral and parenteral administration; (3) isolation and identification of the major drug metabolites; and

STANFORD RESEARCH INSTITUTE (NIH-NCI-C-72-3200) Continued

(4) properties of the enzyme systems responsible for metabolism (subcellular localization, cofactor requirements and susceptibility to known inducers and inhibitors of drug metabolism). Both in vivo and in vitro studies are conducted using at least three animal species, including one species of subhuman primates.

STANFORD RESEARCH INSTITUTE (NO1-CM-33723)

This contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The Contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality of the necessary analytical data for the Food and Drug Administration. This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, liquid and gas), absorption spectroscopy (ultraviolet, visible, and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases, methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (NO1-CM-33742)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids and other areas as may be requested.

The completion of the synthesis of formycin B has opened the path for the synthesis of additional pyrazolo[4,3-d]pyrimidine C-nucleosides. Work is also in progress in the nucleoside area on investigations with both natural and fraudulent bases, and with modifications in the carbohydrate moiety. A considerable program of folic acid antagonists based upon methotrexate is in progress. Exploitation of leads in the indole alkaloid field is being pursued by synthesis of new indole alkaloids. Compounds related to the known indole alkaloids, ellipticine and olivacine, are under investigation. Special attention is being directed to the study of the chemistry of the antibiotics, daunomycin and adriamycin in order to develop agents of greater specificity at the molecular level. Analogs of daunomycin and adriamycin are being prepared and a total synthesis of adriamycin is under investigation. A modest effort is being extended toward preparing simplified synthetic daunomycin analogs that retain only major features of the parent molecule. Also under investigation is the synthesis of a group of compounds designed to intercalate into DNA in the manner proposed for several antitumor compounds and some unusual heterocyclics.

STARKS ASSOCIATES, INC. (NIH-NCI-C-68-1275)

This contract was for the synthesis and/or resynthesis of nucleosides and their intermediates needed to complete structure-activity studies in this area. The major effort was on the resynthesis of designated nucleosides, selected as key compounds for further study by the comprehensive review and structure-activity

STARKS ASSOCIATES, INC. (NIH-NCI-C-68-1275) Continued

evaluation of nucleosides previously carried out. The assignment of compounds for preparation, whether new synthesis or resynthesis, originated with Drug Research and Development. The work on this contract was incorporated into Contract NIH-NCI-C-72-3203 with the renewal of Contract NIH-NCI-C-72-3203 in March 1973 and Contract PH43-NCI-C-68-1275 was terminated at that time.

STARKS ASSOCIATES, INC. (NIH-NCI-C-72-3203)

This service preparative contract supplies Drug Research and Development with compounds required for drug evaluation studies, with 40% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multikilograms.

STARKS ASSOCIATES, INC. (NIH-NCI-C-72-3705)

This is a service contract which supports the Drug Development Branch's efforts in the procurement of chemicals and drugs for the evaluation in the primary screen. The Contractor is required to: (1) provide liaison services to develop new sources of material and to encourage potential suppliers to participate in the program through voluntary contribution of samples; (2) collect compounds; (3) provide support services in the processing of compounds; and (4) aid in the development of methods for selecting materials for the screen.

TACONIC FARMS, INC. (NIH-73-C-912) (formerly NIH-72-C-381)

This contract furnishes approximately 260,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TENNESSEE, UNIVERSITY OF (NIH-NCI-C-70-2084)

The objectives of this contract mainly include formulation development studies and small batch production of oral solid dosage forms. The Contractor also has the capabilities to prepare parenteral dosage forms in pilot batch lots. All work is assigned by the Project Officer for the contract.

TEXAS INBRED MICE COMPANY (PH43-NCI-C-68-88)

This rodent production center supplies a variety of inbred and hybrid rodents for tumor transplantation; for hybrid production (at Texas Inbred and other animal production contractors); and for compound evaluation studies. Animals are supplied from a colony of 25 strains of rodents.

TEXAS INBRED MICE COMPANY (NIH-72-C-375)

This contract furnished approximately 260,000 B6D2F$_1$ (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals were furnished from genetic centers and/or rodent production colonies. This contract termination March 1973.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (PH43-NCI-C-66-1156)

The purpose of this contract is to carry out studies on the pharmacology and physiological disposition of new antitumor agents in man and in experimental animals. Information of potential clinical usefulness is obtained on the absorption, plasma clearance, distribution, plasma protein binding, metabolism and urinary and biliary excretion of such agents. Analytical methodology is developed where not previously available. Where applicable, the development of comprehensive pharmacokinetic models with potential predictive value is carried out. Studies are closely interrelated with clinical trials of new agents also in progress at the M. D. Anderson Hospital and Tumor Institute. The selection of compounds for study is made by the Project Officer following consultation with the Associate Scientific Director for Experimental Therapeutics, and reflects the interests of the NCI's Program in developing new antitumor drugs for clinical trial in man. The data generated from these studies is integrated with data from other in-house and extramural cooperating pharmacology units conducting similar studies with other antineoplastic agents. These data are available to the NCI staff and other interested scientific personnel through the Experimental Therapeutics Master Record, semiannual meetings of the NCI Pharmacology Committee, scientific journal publications and national meetings of various scientific societies.

TEXAS, UNIVERSITY OF (NIH-NCI-C-71-2094)

This contract is designed to assess the value of protected environments in the chemotherapy of acute leukemia using appropriate controls for a quantitative and qualitative comparison. In this study, a protected environment is defined as a laminar air flow room, used in conjunction with an extensive antimicrobial prophylactic regimen developed from previous studies. The ultimate goal of the project is to reduce the risk of severe infection so that more effective chemotherapy can be given.

TEXAS, UNIVERSITY OF (NIH-NCI-C-73-3710)

The objective of this contract is to identify clinical effectiveness of antitumor agents for the treatment of advanced ovarian carcinoma. This common tumor of women is known to be quite sensitive to a variety of alkylating agents as a class. The Contractor will randomize 100 previously untreated patients with Stage III or IV ovarian carcinoma to one of three choices of treatment.

TEXAS, UNIVERSITY OF (NO1-CM-33737)

This is one of three Medical Oncology contracts effective in January 1973 for the conduct of eradicative clinical trials of patients with bronchogenic carcinoma. Comprehensive vertical treatment plans will be adapted to the characteristics of the major types of lung cancer, comparing best available treatment with promising new approaches to therapy.

THOMPSON, JOHN I., COMPANY (NIH-NCI-C-69-2158)

The purpose of this contract is twofold: (1) provides the programming assistance needed to run the drug cost reporting system and continue the development

THOMPSON, JOHN I., COMPANY (NIH-NCI-C-69-2158) Continued

of the clinical drug inventory system, and (2) provides the clerical help needed to maintain current, accurate drug shipment files. These files serve as a record of all drugs sent to investigators by the NCI and include such information as: drug name, NSC number, lot number, formulation, quantity shipped, shipment cost, investigator name, address and group affiliation. Data are derived from bulk chemical and pharmaceutical receipt records and from clinical drug request forms.

TRW, INC. (HAZLETON LABORATORIES, INC.) (NIH-NCI-C-71-2190)

The purpose of this contract is to evaluate the potential use of selected chemotherapeutic agents which may be used intrathecally. This toxicologic evaluation is based upon activity observation, hematology, clinical chemistry, gross pathology, light and electron microscopic examination of animals which have received graded doses of specific chemotherapeutic agents intrathecally. These studies are carried out in close cooperation with the Baltimore Cancer Research Center personnel for purposes of monitoring drug selection and results.

TULANE UNIVERSITY (NIH-NCI-C-73-3701)

This primary genetic center serves to establish foundation colonies of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/L, ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities.

UPJOHN COMPANY (NIH-NCI-C-72-3709)

The objective of this contract is the synthesis of analogs and derivatives of the clinically effective agent, streptozotocin. The major emphasis is on preparing analogs with modifications in the sugar portion of the molecule. Specifically, the Contractor is preparing nitrosomethylureido derivatives of some hexoses, pentoses, tetroses, disaccharides, lactones, glycitols and nucleosides.

UPJOHN COMPANY (NIH-NCI-C-73-3707)

This contract has for its primary objective the development of potentially useful antitumor agents, from fungi, bacteria and actinomycetes fermentation beers, including isolation, purification, characterization, production, biochemistry and pharmacology efforts.

The Upjohn Company has played a leading role in the exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. The present screen consists of the testing of crude and lyophilized beers in a cytotoxic cell test and the actives are then ranked by their cell-cycle specificity. These leads are then tested in-house in the leukemia *in vivo* screen and actives are given top priority.

UPJOHN COMPANY (NIH-NCI-C-73-3707) Continued

Major efforts in this contract are devoted to studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program, and phases of toxicological and pharmacological studies within the capability of the Contractor and requested by NCI.

UTAH, UNIVERSITY OF (NIH-NCI-C-72-3710)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of the antibiotics, tubercidin, sangivamycin and toyocamycin and other nucleoside antibiotics, (2) derivatives of the C-nucleoside formycin, (3) pyrazolo[4,3-d]pyrimidine and pyrazolo[3,4-d]pyrimidine nucleosides, (4) imidazo[4,5-c]pyridine, imidazo [4,5-b]pyridine and v-triazolo[4,5-c]pyridine nucleosides, and (5) imidazole and benzimidazole nucleosides. This Contractor provides these difficult materials in quantities sufficient for in vivo antitumor evaluation by methods of synthesis developed by this group.

VALUE ENGINEERING COMPANY (NIH-NCI-C-73-3706)

Data processing services are provided by this contract to Drug Research and Development. The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch, DR&D (including special queries of the master files via computer); (2) documenting all programs and contractor's procedures for data handling and running computer programs; (3) maintaining the computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) reducing and disseminating information on the receipt of compounds including those of particular interest to the Division of Cancer Treatment program; (5) expanding the present system to incorporate secondary testing not previously stored on the master files; (6) developing new formats and methods of data collection; (7) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data.

VETERANS ADMINISTRATION (NCI-CR-64-4)

This program is a cooperative venture between the Veterans Administration and the National Cancer Institute for the clinical evaluation of potential anti-cancer agents.

VIRGINIA, UNIVERSITY OF (NIH-NCI-C-71-2099)

This contract provides for the fractionation and isolation of active agents from plants with confirmed antitumor activity which stem from the program for screening of crude extracts and are assigned to contract chemists. During the current report period, a number of crystalline materials were isolated and are now undergoing further study at various stages of evaluation. Several compounds are scheduled for clinical trial.

WALTER REED ARMY MEDICAL CENTER (NCI-CR-64-5)

The primary objective of this contract is to provide support for Walter Reed Army Medical Center, Hematology-Oncology Service. This includes collaborative studies with Acute Leukemia Group B and more recently the Medical Oncology area. Protocols are available for patients with acute lymphocytic leukemia, acute granulocytic leukemia, chronic lymphocytic and chronic granulocytic leukemia, Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma. They have contributed a total of over 124 patients to the ALGB.

WARF INSTITUTE, INC. (PH43-NCI-C-66-494)

This is a service contract for the in vivo screening of new, crude plant extracts, fractionations of previously confirmed natural products, new synthetic materials, and follow-up testing of active materials. Currently, testing is being conducted at a level of approximately 50,000 tests per year according to Drug Research and Development protocols. The P388 lymphocytic leukemia is employed as the test system for crude natural products. Those that are active in this system are then tested in the L1210 leukemia. The L1210 leukemia is utilized for the testing of new synthetic materials, with selected materials also tested in the B16 melanoma. Other DR&D tumor systems may occasionally be used in the further testing of materials that were active in those systems or for analogues of such materials. Schedule dependency studies are conducted upon request for materials of potential clinical interest. Methodology and/or research and development, as well as special research programs are scheduled as the need arises and are directed by the Project Officer.

WARF INSTITUTE, INC. (PH43-NCI-C-67-747)

This represents a service contract to prepare 50% aqueous-ethanol extracts of plant material at the rate of approximately 11,000 extracts per year. A semi-quantitative test for alkaloids and tannins has been run on plant materials of sufficient interest to be considered for fractionation. The majority of the Contractor's plants are obtained through a transfer of funds agreement between the NCI and the U. S. Department of Agriculture. During the year provision was made, on a trial basis, for a preliminary fractionation of 500 plants in order to test a procedure for increasing the yield of L1210 active plants.

WARNER-LAMBERT RESEARCH INSTITUTE (NIH-NCI-C-72-3250)

The objectives of this contract are to develop, manufacture and package parenteral dosage forms of investigational drugs for clinical evaluation. The Contractor is responsible for completing required analytical and safety tests on each product prepared. All work is assigned by the Project Officer for the contract.

WASHINGTON STATE UNIVERSITY (NIH-NCI-C-72-2059)

This primary genetic center has as its objectives the development of germfree foundation colonies of inbred rodents. Pedigreed animals are derived via hysterotomy and foster-suckled in germfree isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic

WASHINGTON STATE UNIVERSITY (NIH-NCI-C-72-2059) Continued

organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

WASHINGTON, UNIVERSITY OF (NIH-NCI-C-72-2193)

The purpose of this research-type contract is to explore the effects of high doses of chemotherapeutic agents in conjunction with autologous and homologous bone marrow infusions. Emphasis is being directed to the hematopoietic toxicity and immunosuppressive manifestations of the agents. The investigation involves determining which antineoplastic agents or combination of agents have dose limiting bone marrow toxicity and in fact do not produce significant immunosuppression. In addition, these studies will reveal any unexpected drug-induced toxicity associated with unusually high doses of selected oncolytic drugs.

WEIZMANN INSTITUTE OF SCIENCE (PL-480 -Agreement No. NIH-06-025-1)

The objectives of this contract are: (1) to seek selectivity in cancer therapy through modification of the physical properties of anticancer agents by conjugation with various oligomers and polymers, and (2) to produce polymers of amino acids which in themselves may have anticancer activity.

WISCONSIN, UNIVERSITY OF (NIH-NCI-C-71-2275)

The principal objective of this contract is to study the mechanism of replication of genetic information, contained by RNA-tumor viruses, as well as the mechanism of integration of this information into the host chromosome. To accomplish this objective, studies are in progress with purified enzymes (from avian myeloblastosis virus) in order to understand clearly the enzymic reactions involved. The results on each isolated reaction will be assessed with relation to the physiology and life cycle of the intact virus. The DNA and RNA enzymes have been implicated in the etiology of human cancer. An understanding of the properties and mechanisms of these enzymes is, therefore, fundamental for the eventual solution of this problem. In addition RNA-DNA hybrid template-primers of defined frequence and purity will be prepared and provided to the Project Officer.

WISCONSIN, UNIVERSITY OF (NO1-CM-33733)

This contract, utilizing cell culture techniques, provides a rapid assay as an aid in the purification of potential antitumor agents from plant materials. The materials tested are the fractionation products prepared for the purpose of isolating the active agents from materials having established antitumor activity in the Drug Research and Development in vivo screen. The principal methods used are in an agar diffusion system and bioautography of paper chromatograms.

YALE UNIVERSITY SCHOOL OF MEDICINE (NIH-NCI-C-71-2339)

The objective of this contract is to develop anticancer agents which prevent vital cell function or replication by intercalating into nucleic acids to prevent strand separation. Evaluation of the strength of binding is made by determination of melting temperature and calculation of binding constant. Analogs of a parent compound are made to increase desirable characteristics and minimize unfavorable ones. Various types of nucleic acids are used, such as deoxy-deoxy, ribo-ribo, and hybrid deoxy-ribo strands.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM-33740)

This service-type contract is concerned primarily with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of new drugs. The toxicity is characterized as to predictability, reversibility and schedule dependency. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

YALE UNIVERSITY SCHOOL OF MEDICINE (NIH-NCI-C-73-3711)

The objective of this contract is to provide *in vitro* screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of dihydrofolate reductase from L1210 leukemia cells, from human chronic myelogenous leukemia and from normal red blood cells. Special pharmacologic studies on selected agents are also carried out.

Summary Report
Associate Director for Program
Division of Cancer Treatment

The Division of Cancer Treatment has as its objective the control of cancer through the use of chemicals or chemicals in combination with other modalities of therapy. The Office of the Associate Director for Program has principal responsibility in advising the Director of the Division in planning and formulating logical organization of activities aimed at achieving the objectives of the Division. This responsibility also includes effective coordination and integration of the functions of the various organizational segments comprising the Division with relevant activities of other groups within the NCI and within the NIH as well as with efforts funded under contract with grant supported cooperative groups and with other organizations and institutions.

The National Cancer Act of 1971 and the greater emphasis on a national effort in cancer have brought broader responsibilities to the Division. Where previously the objective of the Division was to develop drugs with effectiveness in the therapy of cancer, its responsibilities have now been broadened to include not only drug development but also the investigation of optimal combinations of drugs, surgery, radiotherapy or immunotherapy with the aim of increasing the incidence of remissions and prolonging disease-free intervals in patients with cancer and the achievement of cures.

In the past, a great deal of effort and resources were devoted to the more rapidly growing neoplasms such as acute lymphocytic leukemia and Hodgkins' Disease. As a consequence, a significant proportion of patients with these types of cancers achieve remissions and life expectancies approximating that of the normal comparable population. From these and other developments, it has become obvious that under most circumstances combination chemotherapy is more effective than single drug therapy and that in appropriate situations, treatment with combinations of modalities provides the optimal approach. Accordingly, the major emphasis is now being placed on the slowly growing tumors such as carcinoma of the lung and of the breast utilizing the concepts which have emerged from the experience with the rapidly growing tumors.

The broader responsibilities of the Division of Cancer Treatment have also brought new problems. Although the Division does not have responsibility for developing research programs in surgery, radiotherapy and immunotherapy, it is essential for the Division to monitor developments in these areas and to coordinate its own activities involving these modalities of therapy with those of the other Divisions having programs in these same areas. To accomplish this a small committee with representation from each of the four divisions has been established to identify informational requirements of each Division and to develop a means of rapidly exchanging such information.

Although the Division of Cancer Treatment now has responsibility for combined modality therapy, its drug development program remains as a focal point for the national effort in this area. The nucleus of this program is at the National Cancer Institute but it is implemented predominantly through efforts supported by contracts with industry, universities, and non-profit organizations. It encompasses every phase of drug research from acquisition of drugs, identification of anti-tumor activity, fundamental research into reversing the neoplastic transformation, pharmacokinetics and its integration with normal and tumor cell kinetics, toxicology, clinical trials, and the dissemination of new information related to cancer chemotherapy.

Program goals and objectives are formulated and implemented by the Division Director and the senior program leaders with a constant effort to predict approaches most likely to result in success in the shortest possible time. Developments in the scientific and medical world are constantly monitored in the search for new potentially important leads which might be exploitable for the control of cancer. One of the most important monitoring activities concerns surveillance for new anti-tumor agents developed by independent investigators both in this country and abroad. To implement this effort the Division has coordinating offices in Tokyo and in Brussels. In addition, the recent agreements in health and science with the U.S.S.R. have already resulted in a number of interesting compounds for evaluation in this country.

These plans formulated by the program leaders are translated into a formal and logical sequence of steps and is embodied into the Program Logic which reflects the scientific basis for the program and at the same time is also and operational plan. The Program Logic has three components: a Linear Array (essential and minimal operational activities required to evaluate new agents), the Supplementary Array (activities contributing to methodological improvements in the Linear Array), and the Exploratory Array (research in relevant areas which if elucidated and exploited might have major impacts in therapy).

The Program Logic is refined and updated continuously consistent with changing plans and changing priorities so as to take advantage of significant scientific developments. Efforts are currently underway to revise the Program Logic to reflect the broader responsibilities of the Division in combined modality therapy.

The budget for the Division of Cancer Treatment is allocated by stages of the Linear Array and by other components of the Program Logic. A computer program is utilized to monitor and assess budget information related to segments of the program and to more specific project areas both in the intramural area and in the contract program. An effort is currently underway to assess funding support in cancer treatment on a national level in view of the national responsibilities of the Division.

The increased emphasis on cancer provides new opportunities to apply the knowledge gained from experience and advances in the management of the

rapidly growing but less common tumors to the slow growing far more frequent tumors. The prospects for achieving great improvements in morbidity and mortality in these tumors in view of the appearance of new drugs such as adriamycin and recent developments in molecular biology and other advances appear to be most promising.

Serial No. NCI- 7085
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 Through June 30, 1973

Project Title: Kinetic (Quantitative) Studies on Tumor Cell and Biomarker Interactions; and on Cell Age, Cell Size, and Cell DNA Distributions and Their Implications to Cancer Chemotherapy

Previous Serial Number: None

Principal Investigator: Kwang B. Woo

Other Investigators: M. Omine, Seymour Perry, M. Kim, and Richard M. Simon

Cooperating Units: Department of Electrical Engineering, Cornell University; and Medicine Branch, NCI

Man Years:

Total: 1.7
Professional: 1.4
Other: 0.3

Project Description:

Objectives

1. To establish a quantitative relationship between tumor cells and their specific biomarkers. A mathematical model is developed which by using the concentration measured of a biomarker and the characteristics by its kinetic parameters, enables one to estimate the total number of tumor cells as well as any variation in number after therapy.

2. To determine the dynamics of cell cycle and proliferation kinetics and the effects of various antitumor drugs. The time course behavior of the cell age, cell size, and cell DNA distributions of a tumor cell population is characterized by a discrete-time system employing computer techniques; and it is compared with experimental results.

3. To study by the formulation of metabolic reaction kinetics inhibitory activities of a number of antitumor drugs on specific enzymes in biosynthetic pathways, and to provide a better basis for optimum schedule of a drug or combinations of drugs for the treatment of leukemias and other forms of cancer.

Methods Employed

1. The proliferation kinetics of the Ehrlich ascites tumor system with an inoculum dose of 4 x 10^6 tumor cells showed growth pattern of a Gompertzian type and cell size distribution of time-varing form (Virchows Arch. Abt. B. Zellpath. 11:1-10, 1972); and changes in the mean concentrations of putrescine, spermidine, spermine, DNA, and RNA were also measured at various intervals after inoculation of the tumor (J. Nat. Cancer Inst. 48:165-171, 1972). With information available on cell cycle kinetics of the Ehrlich ascites tumor and with the assumption (or a positive speculation) that putrescine may be a growth factor, the kinetic relationship between the tumor cells and polyamines was characterized by a system of nonlinear differential equations. Various kinetic characteristics of tumor cell proliferation and polyamine synthesis were studied by computer simulation and subsequently evaluated with the available experimental data.

2. The dynamics of cell cycle kinetics in reference to L1210 leukemic mice cells were formulated by first order nonlinear differential equations of the cell balances in each cell cycle phase, including G_o-phase for the nonproliferating cells. With the specific values of kinetic parameters, the mean cell cycle time and the rate of cell loss (Cancer Res. 29:1581-1584, 1969; 32:2452-2458, 1972), the patterns of tumor cell growth in each cell cycle phase and the effects of the variation of cell loss were studied. Two kinetic parameters were specifically defined: the transit time that represents the time of a cell residing in a compartment, and the transfer factor (K_m) that represents the relative rate of cell movement in a tumor cell system.

3. The activities of polyamine-synthesizing enzymes and endogeneous polyamine concentrations in L1210 leukemic mice cells (Cancer Res. 33:159-163, 1973) were described by the Michaelis-Menten reaction kinetics, polyamine catabolism and other losses, and also in terms of individual cell growth and mitosis. Information used on the enzyme reaction kinetics of polyamine syntheses was previously obtained (Adv. Enzyme Regul. 7:291-324, 1969; 10:225-245, 1972). A quantitative relationship was established between cell cycle kinetics and polyamine concentrations in reference to L1210 leukemic mice cells, and was used to evaluate the effects of methotraxate and 5-azacytidine.

4. Under conditions of unlimited growth, a discrete-time system model was developed by using the state equations which are linear difference matrix equations with its state vector consisting of variables representing cells in a particular compartment for cell age, cell size, and cell DNA content. The time course behavior of the cell size distribution of labelled cells under a flash labelling was investigated by computer simulation and compared with the experimental results (J. Nat. Cancer Inst. 48:697-704, 1972) obtained for spontaneous AKR leukemic cell population.

5. The relative activities of allosteric enzymes involved in branched biosynthetic and amphibolic pathways were studied by the formulation of the control characteristics as a function of activitor and inhibitor. Attempts were made to use this approach to the analysis of combinations of drugs inhibiting steps in the biosynthesis of cofactors or nucleic acids, which are described as sequential blockade, concurrent inhibition, and complementary inhibition (Adv. Enzyme Regul. 10:63-80, 1972).

Major Findings

1. Tumor Cell-Biomarker Kinetics. A model based on three compartments - the proliferation and the nonproliferation of tumor cells and the synthesis of biomarkers - was developed to represent the dynamics of tumor cell and biomarker interactions. A system of nonlinear differential equations derived for the model includes two kinetic parameters for the control of tumor cell proliferation, self-inhibition and stimulation. The main characteristics of these parameters are basically a cooperative control in nature which displays strong sigmoid response to the number of tumor cells and the concentration of markers. Implementing the experimental data obtained from cell kinetic studies, a quantitative relationship between Ehrlich ascites tumor and polyamine (putrescine) was well demonstrated by the proposed model.

The tumor cell balance equations and the specific enzymic reaction kinetics for five cell cycle phase compartments, G_1, s, G_2, M, and G_o, were developed and characterized by computer simulation. Considering one separate compartment which maintains 10^6 cells throughout the cell cycle period, the steady state value of each polyamine concentration was determined by its initial value. The result also illustrated that the decay rate for all polyamine is too low for the selected V_{max} values, which brings about the evidenced increased in polyamine concentrations throughout the entire period of tumor life. The variation of cell loss and its effects on the patterns of tumor cell growth in each cycle phase indicated that the effect of increased cell loss in S-phase is most significant in which the tumor growth becomes negative and the tumor size decreases after an initial small increase.

2. Cell Age, Cell Size, and Cell DNA Distributions. In discrete-time system model formulated for the dynamics of cellular proliferation, cell size distribution in a cell culture is obtained from the knowledge of cell age distribution by a linear transformation. The linear transformation was based on a hypothetical curve for the growth in mean size of a single cell as it matures and also on the assumption that the size distribution of the same age is Gaussian with known coefficient of variation. Cell DNA distribution in a cell culture consisting of one type of cells can be estimated by the knowledge of cell age distribution. In this case, a hypothetical curve for the rate of DNA synthesis of a single cell during the S-phase was tired for the linear transformation, which is compared well with experimental observations.

The model can be used to determine theoretically the time course of the cell age, cell size, and cell DNA distributions of a cell population and the correlation of these cell kinetic parameters under various environmental conditions. The movement of the cell size distribution of labelled cells under a flash labelling revealed the closely resembled pattern as shown by spontaneous AKR leukemic cell population studies.

3. Metabolic Control Mechanisms. The study on the simple end product inhibition with the regulatory function of a saturation type nonlinearity showed that the piecewise linearization method for the nonlinearity provides more accurate solutions of metabolite oscillations, while the optimal linearization method is very effective in obtaining quantitative information on the oscillatory conditions.

A kinetic model of the compensatory feedback control in reference to E. coli carbamyl phosphate synthetase yielded sustained oscillations as well as the ampitude-modulated oscillations. In characterizing the nonlinear feedback function of the regulatory enzyme involved, a two-input (activator and inhibitor) nonlinearity was approximated by two single-input nonlinearities. One of the significant features of this result was that the nonlinearity reveals a memory type characteristic, which is long suspected in enzyme actions.

Significance to Biomedical Research and the Program of the Institute

A mathematical model developed for tumor-marker interactions characterizes well the kinetic relationship between Ehrlich ascites tumor cells and polyamine (putrescine). The clinical implications of the model would be greatly enhanced when the size or proliferation rate of tumor is estimated by this model using urinary polyamine in patients with various forms of cancer and leukemias. The model may be applicable to other types of tumor cells and corresponding biomarkers, which requires different functional forms of kinetic parameters that represent the control of tumor cell proliferation.

A discrete-time system model can describe and predict the time course behavior of the cell age, cell size, and cell DNA distributions and also the movement of labeled cohort through the cell cycle. Such characteristics are useful to gain insights into the dynamics of cell cycle and proliferation kinetics and the effects of antitumor drugs.

Proposed Course

1. Efforts are presently being made to integrate the proliferation kinetics of L1210 leukemic mice cells and the activities of enzymes responsible for polyamine syntheses, and to characterize the effects of antitumor agents, 5-azacytidine and methotrexate, using experimental data.

2. The sensitivity analysis of kinetic parameters included in the tumor cell balance equations will be carried out with respect to the variation of tumor cells and polyamine (or other marker) concentrations.

3. Specific efforts will be made to formulate and modify this model to allow its future extension to other types of biomarkers for cancer. Different (new) hypotheses and assumptions will be proposed and investigated in relation to tumor growth mechanisms as a function of tumor cell size and substances responsible for cellular regulation.

4. The discrete-time system model will accommodate both the proliferating and the nonproliferating compartments and also the factor of cell loss. The effects of antitumor agents on the cell size and cell DNA distributions will be investigated by including a state vector feedback in the model, which then becomes a discrete-time feedback control system. Analytical techniques developed on discrete-data control systems will be applied to study: (a) the stability of the system to determine the time course behavior and various relations of kinetic parameters to a drug or combinations of drugs, and (b) optimal or suboptimal schedule of drug treatment.

5. An attempt will be made to develop a kinetic equation which characterizes inhibitory activities of rifamycin on DNA polymerases (J. Nat. Cancer Inst. 49:7-25, 1972). Patterns of the interaction of drugs that inhibit steps of biosynthetic pathways of nucleic acids will be analyzed; and kinetic studies of the interactions of two antitumor drugs will be carried out in relation to the growth of L1210 in vitro and the rate of DNA synthesis.

Honors and Awards:

Kwang B. Woo

1. Invited by the Department of Biology, University of Toledo and the Department of Physiology, Medical College of Ohio to present a seminar and a graduate lecture on "Dynamics of Tumor-Marker Relationship: A Quantitative Analysis." June 1972.

2. "Use of Polyamine Concentrations as a Diagnostic Tool in Cancer Detection." Presented at the 1972 International Conference on Cybernetics and Society in Washington, D.C. October 1972.

3. "A Quantitative Model for Relating Tumor Cell Number to Polyamine Concentrations." Presented at the National Cancer Institute Symposium on Polyamine-Cancer in Hunt Valley, Maryland. October 1972.

4. Invited by the Program on Science, Technology and Society, Cornell University (Dr. F. A. Long) to participate in a Workshop on Cornell-KAIS (Korean Advanced Institute of Science) Collaborative Activities to discuss selected policy problems for science and technology in developing nations and collaborative efforts in teaching and research. November 1972.

5. Elected to IEEE Representative to American Automatic Control Council (an association of Am. Inst. Aero. Astro., Inst. Electrical & Electronics Engr., Am. Soc. Mech. Engr., Am. Inst. Chem. Engr., Instrument Soc. Am.): Member, the Biocontrol Committee, 1973.

Publications:

1. Woo, K.B.: Control characteristics of regulatory enzyme systems, I. Nonlinearities of regulatory enzyme activities. Math. Biosci. 13:9-13, 1972.

2. Ghista, D.N., Patil, K.M., Woo, K.B., and Oliver, C.: A human left ventricular control system model for cardiac diagnosis. J. Biomechanics 5:365-390, 1972.

3. Woo, K.B., and Simon, R.M.: Use of polyamine concentrations as a diagnostic tool in cancer detection. Proc. 1972 Internatl. Conf. Cybernetics & Society, pp. 88-93, 1972.

4. Ghista, D.N., and Woo, K.B.: Computerized cardiac monitering and simulation modeling in cardiac diagnosis. Chapt. 6. In: Computer Techniques in Biomedicine, Haga, E. (ed.), Philadelphia: Auerbach, pp. 121-148, 1973.

5. Woo, K.B., and Simon, R.M.: A quantitative model for relating tumor cell number to polyamine concentrations. In: Polyamines in Normal and Neoplastic Growth, Russell, D.H.(ed.), New York: Raven Press, pp. 381-393, 1973.

6. Ghista, D.N., Patil, K.M., Gould, P., and Woo, K.B.: Computerized left ventricular mechanics and control system analyses models relevant for cardiac diagnosis. Comput. Med. Biol., in press.

7. Woo, K.B., and Huang, H.M.: Compensatory feedback control in biochemical reactions: control activity of E. coli carbamyl phosphate synthetase. Proc. IFAC Symp. Dynamics & Control in Physiol. Systems, in press.

Serial No. NCI-6401
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 Through June 30, 1973

Project Title: (A) Data Processing Systems for Scientific and Management Information in the Division of Cancer Treatment
(B) Analysis of the Linear Array: Computer Simulation of a Stochastic Model; and Quantitative Formulation of Scheduling and Operation

Principle Investigators: Meyer H. Gordon, Leon Rothenberg

Other Investigator: Kwang B. Woo

Cooperating Unit: DCRT

Man Years:

Total: 2.0
Professional: 1.6
Others: 0.4

Objectives

1. Establish a complete file management system for the classification of DCT expenditures by stage of program logic, basic priority stratification of the DCT program, and National Cancer Program objective (Approach 6).

2. Analysis of the Linear Array by the development of quantitative models to be used as an aid to the management decision-making process in the scheduling and operation of the overall DCT program.

3. Coordinate data pertaining to computer-oriented expenditures within the Division of Cancer Treatment.

4. Develop a system to be used for the storage and retrieval of scientific information by cross-referencing between disease and drug categories.

Methods Employed

1. Through the use of DCRT program generation software, a single file containing all DCT Contract and Schedule A data is updated monthly at the terminal in the conversational mode. This update is designed for the use of non-programmers. All entries in the file are classified with respect to stage of program logic, basic priority stratification of the DCT program, and National Cancer Program objective (Approach 6).

2. The formulation of a mathematical model of the Linear Array is presently under way to investigate scheduling and operation under parametric perturbations. In stochastic analysis, the Linear Array is being studied with the generation of arbitrary random variables as input to the model.

3. With the cooperation of the Administrative Officers of DCT, data pertaining to DCRT accounts for DCT is now coordinated by this office.

4. Work has begun on the development of a system that is disease oriented, cross-indexed to single and combination agents, easily updated, and containing readily retrievable information. A mockup of such a file with appropriately indexed entries has been prepared.

Major Findings

1. Programs have been written by DCRT to generate reports by various combinations of the three classifications applied to the Contract and Schedule A data, as well as by DCT organization.

2. A preliminary model of the scheduling problem has been charted, and the detailed analysis of the model is now under study. A very basic stochastic model has been programmed and is in the process of modification and expansion.

3. The availability of contract and DCRT account data have enabled this office to formulate the annual ADP plan requested by the OD, NCI.

Significance to the Program of the Institute

Classification of the Contract and Schedule A data by stage of program logic, subclassification, and NCP approach category with the subsequent generation of reports using programs written at DCRT has led to a detailed description of the DCT research program. The establishment of quantitative models of the Linear Array will enhance the program analysis and planning of DCT and also aid in the decision-making process of DCT management, leading to optimum allocation of resources.

Proposed Course

In cooperation with DCRT, additional report generating programs will be written to supply more information concerning the allocation of DCT funds. Direct Operations data will be added to the computer file in the new future. As the quantitative models are developed further, there will be interaction with the various units of DCT in an effort to validate what has been established as well as to modify and expand these models.

Honors and Awards

None

Publications

None

Serial No. NCI- 7025
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 Through June 30, 1973

Project Title: Biomedical Engineering Resources for Intramural Research, DCT

Previous Serial Number: None

Principal Investigator: William Z. Penland

Other Investigators: NCI: Kwang B. Woo, Seymour Perry, Charles E. Leasure, Stanley E. Shackney, Lewis E. Lipkin, Arthur S. Levine, David Poplack, Vincent H. Bono, Robert E. Gallagher, Robert G. Graw, Joan M. Bull, Malcolm I. Bull, Peter Lohrman, Jeoffrey Herzig, Philip T. Waalkes
ADL: Alfred E. Wechsler, Leonard Peusner, Phillip S. Thayer, Philip G. Drew, Edward J. Cook, Erica L. Drazen, Sandra Harris, Donald B. Lindsay, Robert H. Liss, Karl M. Wiig, Bruce Shapiro, Jack Ennis
RTI/NASA: F. Thomas Wooton
ORNL: Julian P. Breillatt, Norman G. Anderson
ABCOR: Richard de Fillipi, Grady Harris, Richard Anderson
NBS: Russell A. Kirsh, Philip G. Stein

Cooperating Units: Division of Cancer Treatment Program Staff; Office of the Associate Scientific Director for Program, DCT; Medical Oncology, DCT; Experimental Therapeutics, DCT; Drug Research and Development, DCT; Baltimore Cancer Research Center, DCT; Nuclear Medicine Department, Clinical Center, NIH; Research Triangle Institue; Oak Ridge National Laboratories (ORNL); Arthur D. Little, Inc. (ADL); Sci-Med, Inc.; Abcor, Inc.; National Bureau of Standards (NBS); NASA, Goddard Space Flight Center.

Man Years:

Total: 16.5
Professional: 15.5
Others: 1

General Objectives/Scope

1. To provide staff engineering capability augmented where necessary by specialized or broadly based biomedical engineering resources which are capable of direct support to diverse engineering needs of individual research projects within the Division of Cancer Treatment.

2. To test and refine an effective management approach concerned with focusing competent bioengineering resources via the contract mechanism to define and implement key bioengineering projects that are responsive to the changing priorities of intramural research programs.

Major Findings/Accomplishments

During 1972 a contractural effort was completed which now provides close and long term Biomedical Engineering (BME) support for the diverse, interdisciplinary needs of intramural research in the Division. A.D. Little, Inc., with unusually broad BME resources that include a staff of 700 professionals, was chosen from a nationwide response of 128 interests in which three (3) were from national laboratories, twenty-one (21) from non-profit organizations, thirty-two (32) from academic institutions and sixty (60) from industrial sources.

A pilot study to use this resource flexibly and effectively with the senior investigative staff has resulted in the definition and initiation of approximately fifteen (15) new BME projects and the completion of twenty (20) cases of technical consultation during this reporting period. The DCT Program Staff monitored all initial engineering activities through periodic reviews and approval of defined projects. The impact and the integration of these activities upon the research and clinical investigations of the Division are reported below with principal results of other cooperating units.

Leukocyte Procurement

Two conceptually new and different systems for the efficient procurement of human granulocytes were conceived and proposed during 1972. Both systems will serve NCI investigations (Dr. Graw et al) and are mechanically compatible with all manufactured blood cell separator units now representing approximately a one million dollar investment outside the NCI. ABCOR engineers completed a theoretical analysis, mechanical design, and fabrication effort for a continuous centrifugal elutriator (rotor) theoretically capable of 80% granulocyte recovery with 3% red cell loss. The rotor is scheduled for initial trials with dogs in June 1973.

ORNL engineers completed a comprehensive critical analysis of all granulocyte isolation methods during this period with the conclusion that leukoadhesion and centrifugal separators, exploiting the differential sedimentation of granulocytes and red cell rouleaux, would offer the greatest probability of yielding a successful in vivo leukapheresis device. Accordingly, an ORNL proposal to develop a rotor based upon the above findings was favorably reviewed this period and awaits '74 funding. The rotor design uniquely incorporates a number of

rouleaux enhancement techniques together with a photoelectric sensor that promises to continuously and automatically monitor and control the separation of newly formed red and white cell layers within the rotor cavity. Granulocyte yields are expected to approach 50%.

Some eighteen (18) in vitro and in vivo runs with dogs using a prototype device, the NCI/ABCOR Interface Controller, have established the utility of the device in controling the hemoglobin content of the buffy coat collection automatically and continuously during separator collection periods of 2-4 hours within a range of 2-4 gram percent hemoglobin that otherwise is manually not obtainable. Improved operator convenience and buffy coat yields are expected from current human trials of the controller device.

Engineering support of NCI studies (Graw, et al) using various filtration leukapheresis techniques for improved procurement of functional granulocytes now consists of ADL projects to characterize the morphological/topographic relationships between filter matrices of both adherent and isolated granulocytes using SEM techniques and to also provide appropriate filter materials (glass microspheres, etc.) and engineering consultation.

Mathematical Modeling

New efforts during this period were directed to the development of mathematical models for tumor cell kinetic and biomarker activities (Dr. Woo, Mr. Wiig). The problem of tumor cell-marker interactions is being investigated by the development of a kinetic model to estimate and monitor by measurement of a biomarker the numbers of tumor cells and their variation after therapy. The Ehrlich ascites tumor-polyamine system has satisfactorily been formulated and characterized by experimental data available. Further attempts are presently being made to establish a quantitative relationship between cell cycle kinetics and polyamine concentrations in reference to L1210 leukemic mice cells, and to use this relationship to evaluate the effects of methotraxate and 5-azacytidine.

In cell cycle kinetics studies, the dynamics of cell cycle and proliferation kinetics are characterized by the state equations which are linear difference matrix equations with its state vector consisting of variables representing cells in a particular compartment for cell age, cell size, and cell DNA content. The time course of the cell size distribution of labelled cells under flash labelling was investigated by computer simulation and favorably compared with the experimental results obtained for spontaneous AKR leukemic cell population (J. Nat. Cancer Inst., 48:697-704, 1972).

Protective Environment Study

Engineering support of the protective environment study (Levine, et al) was furnished through several ADL projects and consultation. A unique prototype bacterial isolation garment, affording mobility to the patient for medical and psychological reasons, has been developed and tested in rapid response to patient requirements. Penetration tests using di-octyl phthalate and fluorescent zinc cadmium sulfide particles have demonstrated the effectiveness of the basic design and construction based upon the use of positive pressure, high efficiency air filtration and a NASA fabric of minimum biological penetration characteristics. The suit is now undergoing tests in the clinical environment.

Another ADL project was designed to complement the NCI clinical assessment of the protective environment (Levine, et al), New England Journal of of Medicine, 288:477-483, March 8, 1973) through an independent engineering evaluation of the non-clinical aspects of operating laminar flow rooms in four (4) NCI sponsored clinical trials. Intramural and published reports from this effort, should provide a state-of-the-art summary of the implementation of laminar flow rooms (past performance and future requirements particularly with regard to staffing) in enough detail to aid realistic early planning efforts by interested institutions.

Automatic Image Processing

Substantial engineering support for the NCI investigation of automatic grain counting and blood cell identification (Lipkin, et al) from the National Bureau of Standards was converted in part during 1972 to specific projects and tasks of the ADL contract. NBS accomplishments included a variety of support tasks involving the processing of scanned images of cells, experimentation with computer algorithms for image analysis, grain counting and instrument controls, and provision of engineering assistance in the development and interfacing of software and hardware components of NCI laboratories conducting the primary investigations. An ADL analysis and evaluation of a computer controlled acousto optic filter for an advanced microscope system was completed and follow-up hardware procurement initiated. Another ADL project was directed to evaluate the most desirable artifical intelligence system and related software for use with the automated microscope and computer hardware for the identification of human blood cells. ADL consultation was provided to determine the feasibility of developing a controlled micro-environment chamber compatible with Dr. Lipkin's advanced microscope system.

NASA Technology Developments

NASA technology transfers and engineering assistance through Dr. T. Wooten, RTI, during this period have focused upon three areas:

(1) A novel freeze/thaw apparatus, applicable to in-house cryopreservation studies of white cells and bone marrow (Dr. M. Bull), was conceived at the Jet Propulsion Laboratory, NASA and is under NASA sponsored development at Goddard Space Flight Center. The prototype in initial tests promises more precise and responsive control of freezing rates than present NCI equipment.

(2) The search for new detection/collimation designs for the scanning of small tumors in animals (a possible adjunct to conventional means of detecting tumor growth and regression in current drug screening studies) has met with some success following reviews of advanced gamma camera proposals from several sources during this period. A novel multi-wire proportional counting chamber (NASA/Ball Brothers Research, Inc.) appears promising for detecting the position of ionizing particles with spatial resolution of approximately 1 mm using low energy radioisotopes. A technical proposal addressing the feasibility of small animal and whole body scanning has been encouraged by the Nuclear Medicine Staff, Clinical Center and will be jointly considered for NASA and NCI funding.

(3) Provision of an Apollo 11 Biological Isolation Garment and technical assistance by the NASA, Houston Life Sciences Division, influenced the suit design and the use of a low bacterial penetration fabric used on the NCI garment developed recently for leukemic patients.

Miscellaneous BME Projects and Consultation

An ADL evaluation of advanced electron microprobe techniques was completed to assist molecular hybridization experiments in the Laboratory of Tumor Cell Biology (Gallagher). Considerable undeveloped potential appeared to exist for probing tracer-element concentrations in moderately thick ($\sim$1μm) cell sections and possibly even in whole cells. Using presently available instrumentation, it was determined that the limit of sensitivity for the detection of iodine, employing a spatial resolution of 100 - 150A°, to be about 1×10^4 atoms (3×10^{-18}g of iodine). Predictions of improvements in equipment performance within the next few years should extend the absolute sensitivity limit to about 1×10^3 atoms of iodine.

An ADL project was initiated to study the feasibility of improved counting of bone marrow colonies to assist the intramural evaluation of hemopoietic stem cell function (Carbone, J. Bull, et al). Automatic (low fatigue), highly reproducible counting of unstained colonies in existing media preparations has been emphasized. A range of sophisticated and simple instrumental techniques are being examined with a view toward machine adaptability to colony counting criteria. Colony counting using automatic image analysers sacrifice accuracy and resolution in counting and their reliability in clinical use is questionable. Improvements will depend upon control of artifacts in existing media preparations and improvements in TV camera discrimination between artifacts and cells. Preliminary results with stereo zoom microscopy using special lighting techniques and the application of an optical beam scanning projector appear to facilitate high resolution manual counting while reducing elements of eye strain and fatigue.

An ADL project was undertaken to prepare uniformly fluorescing particles in the range of 1 to 10 microns diameter to be used as a reference standard in cytofluorimetry studies (Dr. Bono) to determine the size and DNA content distribution of cells. The ADL project investigator concurrently sought available products and investigated the bonding of a fluorescing salt to beads by ion exchange. A product was located and tested successfully thereby terminating a series of planned investigations.

A current ADL project will develop the means and plans for better space utilization of NCI facilities within the Clinical Center, NIH. Initially, a consolidated graphic display of all NCI space, its functional classification, and personnel and equipment densities will be developed so that this information can be viewed upon an isometric enlargement of all 14 floors of Building 10. Such a display can facilitate the identification of severe problem areas and options for space consolidation which will then be analysed and/or planned in subsequent ADL activities.

Additional short term consultation to the NCI staff was provided by ADL specialists regarding the following subjects: State of the Art Evaluation of Existing Cell Sorter Devices; Review of the Micro-channel Plate Amplifier for Radioautographic Grain Counting; Selection of Interim TV System for Radioautographic Grain Counting; Evaluation of Machining Capabilities, Costs, etc. for Automatic Scanning System; Specification of Monitoring Instrumentation for Laminar Flow Intensive Care Unit; Modification of Cytofluorimetric Techniques for Drug Screening at the Cellular Level; and Evaluation of Instrumental Techniques for Biomarker Detection.

Significance

To supplement existing engineering staff and contract support, a broad range of outside biomedical engineering experience and expertise is now available to DCT investigators in a convenient way. Short term feasibility/evaluation type projects between ADL engineers and NCI investigators on a variety of subjects have predominated in the first year of this pilot effort. The majority of these projects are directly responsive and useful to immediate investigations. As in the past, such projects are expected to generate new leads for the characterization and solution of more complex problems, within the Division, that lend themselves to bioengineering attack.

Technical reports are compiled on all biomedical engineering projects and consulting activities primarily for the use of the intramural staff. Publications, honors and awards, lectures, etc. resulting from these efforts generally are reported under the individual research projects.

Proposed Course

Future activities will involve the completion of ongoing projects previously discussed and particularly will determine the feasibility of two (2) different rotor systems for the procurement of human granulocytes in high yields. Future activities will attempt to broaden discussions with investigators in different program segments and explore projects in forseeable areas of interest such as: the feasibility of drug delivery systems involving controlled drug delivery through implantable and intravenous devices; the application of new cell culture devices to the quantitation of biomarker/tumor cell activity and possibly to drug screening studies at the cellular level; the extension of engineering characterization studies using scanning electron microscopy and improved high resolution microscopy techniques; the extension of tumor/biomarker models developed for the polyamines to models of other biomarkers of malignancy; the examination of analytical computer techniques to forecast drug activity. Considerable future effort will be devoted to the rapid provision of unique prototype instrumentation in direct support of program interests in, automatic grain counting and microdensitometry, rapid cell sorting, and bone marrow colony counting.

Serial No. 3409
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Leukocyte Kinetics and Characteristics of Cell Proliferation

Principal Investigators: Mitsuhiro Omine and Gregory Sarna

Other Investigators: Seymour Perry, and Jacqueline Whang-Peng

Man Years:

Total: 3.1
Professional: 2.1
Other: 1.0

Objectives:

1. To investigate the proliferation and cell cycle characteristics of leukemic blast cells in patients with leukemia and in experimental animals in an attempt to provide a firmer theoretical basis for devising more effective therapy.

2. To define the characteristics of the various phases of the cell cycle with emphasis on the resting cell (G_o).

3. To evaluate the effect of anti-tumor agents on normal and tumor cell population kinetics.

Methods Employed:

1. Leukemic or other cells from animals or man are separated, principally on the basis of size, in a special chamber containing a density gradient (sucrose). Separated cells are studied for DNA content, uptake of tritiated thymidine, and proliferative capacity as determined by transplantation and other techniques.

2. Peripheral leukocyte kinetics in patients are studied with the use of $Na_2\ ^{51}CrO_4$ and $DF^{32}P$ as labels in vitro. Following infusion of labeled leukocytes, specific radioactivity is monitored.

3. Surface scanning over organs in patients with a dual collimated scintillation probes is utilized to study organ distribution of infused 51-chromate labeled cells.

4. Tritiated thymidine is administered intravenously in man or animals to investigate kinetics of cellular proliferation of both normal and tumor cell populations.

Major Findings

1. Spontaneous AKR leukemia (mice) is considered a useful experimental model for human acute leukemia because it is similar to the human disease in its marked cell size variation, low growth fraction, and response to cytotoxic agents. Utilizing a sucrose gradient at 1g in the separation chamber, a cytokinetic analysis (based on cell size) of the leukemic thymus cells was performed.

The small cells were not in DNA synthesis and had a normal complement of DNA. The large cells had twice the normal complement of DNA and the majority were in DNA synthesis. Between the extremes of cell size, there were intermediate values of DNA. However, all cells were capable of inducing leukemia in young AKR mice, indicating that the small cells were in G_o and that they were readily capable of recycling.

The cell cycle time is approximately 18 hours; T_S is 7 hours; G_1 + 1/2M, 9 hours; and G_2 + 1/2M, 2 hours. The proliferative fraction is approximately 75% and the cell loss factor is calculated to be 0.80.

2. These small cells were derived from rapidly proliferating large cells and appeared to be in a prolonged G_1 or resting phase. However, the small cell population appeared to be constantly renewed but a significant fraction were considered non-viable. This continual transition of cells between compartments may represent one of the major modes of cell death, i.e., age related within the tumor cell population.

3. In contrast to the larger cells, the small cells were relatively resistant to various cytotoxic agents including cyclophosphamide, vincristine, prednisone, and BCNU.

4. Following treatment with Palm0-ara-C (depot form of arabinosyl cytosine), medium and large cells were preferentially eliminated but a relatively large number of small cells survived. These small cells were still capable of inducing leukemia after 2 days of treatment. However, after 3 days of treatment, this capability was lost. It was concluded therefore, that the small cell population consists of both cells capable of re-entering the proliferative pool and of cells in an end stage phase.

Significance

As indicated above, the spontaneous AKR mouse leukemia is a good experimental model for human acute leukemia since it is similar from a cytokinetic standpoint and in its response to therapy. Since the small cells in human acute leukemia appear to be analogous to the small cells in AKR leukemia and may be responsible for relapse after apparently successful therapy, it is important to attempt to delineate their cytokinetic characteristics in the mouse leukemia so that better results can be achieved in the human disease.

Proposed Course of Project

1. Studies of the effects of anti-tumor agents in spontaneous AKR will be continued.

2. Studies of cell cycle kinetics in human acute leukemia using the special chamber technique will be continued.

3. It is hoped to extend these studies to patients with solid tumors.

Honors and Awards

1. Omine, M. and Perry, S.: Cytokinetic Characterization of Small Cells in Spontaneous AKR Leukemia. Presented at Annual Meeting, American Society of Hematology, Hollywood, Florida. December 1972.

2. Perry, S.: Leukocyte Kinetics. Presented at the 14th International Congress of Hematology, Sao Paulo, Brazil, July 1972.

Publications

Spivak, J.L. and Perry, S.: Evaluation of ^{51}Cr as a Leukocyte Label. British Journal of Haematology, in press.

Perry, S.: Chemotherapy: A Broad Overview. Presented at the National Cancer Conference. Cancer, in press.

Perry, S.: Clinical Use of Leukocyte Preparation. In Williams W.J., Beutler, E., Erslev, R.J. and Rundles, R.W. (Eds.): Hematology. New York, McGraw-Hill, 1972, Chap. 167, pp. 1321-1324.

Perry, S.: Preservation of Leukocytes. In Williams W. J. Beutler, E., Erslev, R.J., and Rundles, R.W. (Eds.): Hematology. New York, McGraw-Hill, 1972. Chap. 163, pp. 1303-1305.

Perry, S.: Leukocyte Kinetics. Brazilian Journal of Medical and Biological Research. 5(5-6): 271-276, 1972.

Serial No. NCI- 7071
1. Office of the Associate Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 Through June 30, 1973

Project Title: Automatic Cell Scanning

Previous Serial Number: NDS (CF)-65 PR/P 1278
(Biologic Pattern Data Processing)

Principal Investigators: Lewis E. Lipkin, M. D.
Peter F. Lemkin

Other Investigators: George Carman
Bruce Shapiro
Morton Schultz
Bernice S. Lipkin, Ph.D.

Cooperating Units: Section on Technical Development, IR M
Computer Center Branch, CR

Man Years:

Total:	1.55
Professional:	1.00
Others:	0.55

Note: Work on this project was largely deferred in order to complete the grain counter within the scheduled time. This delay was largely occasioned by shortages in personnel.

Project Description:

The construction of an advanced "designer;" a general purpose apparatus to allow the on-line investigation of the morphological properties of a class of related biological images such as the cells in a marrow smear. The object of such investigation is to develop an optimal set of properties and morphologic relationships which will allow the automatic classification of members of the classes, their quantitation and characterization.

The hardware components of such a designer of necessity include:

1. An advanced, fully automated research microscope including a high speed digital stage.

2. A random access wavelength control interfaced with a suitable high intensity, ultra-stable light source.

3. Special purpose TV scanning/quantitation (microdensitometer).

4. General purpose computer and peripherals (minicomputer).

5. Suitable interface to control items 1-4 inclusive.

6. Special purpose hardware to allow real time processing of multilevel grey scale pictures.

7. Large general purpose computer (time shared).

The corresponding software includes:

8. At top level a "model" - essentially a generative, logical structure embodying the expressible and deducible properties of the class of cell images.

9. A group of sequencing programs which essentially serve to control the special purpose picture processor.

10. Software for the human (computer mediated) control of the microscope, light sources, wavelength and TV data acquisition system.

11. The ability to conduct data transactions and transfers between the picture system (1-6) and the large computer (7). For this a language such as SAIL (Stanford Artificial Intelligence Language) or similar environment is necessary.

Progress and prospect: Item 1 has been specified. Item 2 has been deeply explored with the assistance of Dr. Cooke, et al of Arthur D. Little with procurement expected during FY 1974. Items 3 and 4 are in process of procurement. Items 5 and 6, now being designed, will be in construction during the entire period of FY 1974 and although partially complete at the end, will be operational. Additional modular functions should be added during FY 1975 and 1976. Item 7 is now available to us in the form of time shared use of the DCRT PDP-10, especially with the use of high speed direct lines multiplexed by a PDP-11. Item 8 exists in skeleton form as "CELLWORLD" - a framework of a formal description of white blood cells. Items 9 and 10 are planned. On the basis of experience with the grain counter, initial versions should be possible within 12 to 18 months, i.e. coincident with the beginning viability of the picture processor. Item 11 is presently the subject of intensive study by an A.D. Little consultant.

Honors and Awards: None

Publications: None

Serial No. NCI- 7070
1. Office of the Associate Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 Through June 30, 1973

Project Title: Automated Grain Counting/Densitometer

Previous Serial Number: NDS (CF)-65 PR/P 1278
(Biologic Pattern Data Processing)

Principal Investigators: Lewis E. Lipkin, M. D.
Peter F. Lemkin

Other Investigators: George Carman
Morton Schultz

Cooperating Units: Section on Technical Development, IR M
Computer Center Branch, CR

Man Years:

Total:	5.8
Professional:	2.8
Others:	3.0

Project Description:

The objective was to construct an instrument which would allow a user (technician or physician) to classify cells in radioautographs and have automatic nuclear and background grain counts performed for each cell so classified. The project involved the interfacing of some commercially available quantitative television microscopy equipment, a general purpose computer and its peripherals, and some specially designed illumination, microscope and general optical equipment. In addition to hardware construction, something in the region of 25,000 lines of programming was necessary to complete the facility for a habitable human use of the equipment.

The project was essentially complete as the fiscal year ended. The apparatus, still to be optimized is operational. Remaining work to be performed includes:

1. Full generalization of the "classification" algorithm which enables the final distinction between the classes of nuclear and non-nuclear grains.

2. Completion of user's manual and hardware specifications (drawings, wiring diagrams, etc.). These should be ready for submission by the end of the second month of FY 1974.

3. Setting up mechamisms for full field trial of replicated and repackaged apparatus including training of individual(s) for hardware/software maintenance.

4. Preparation of patent application for the interface. The apparatus besides representing a grain counter of wide patented versatility, is also with additional programs and software restructuring a first cut at a generalized cytologic measuring device. Within a variable part of the TV field, basic parameters such as area, intercepts, number of discrete detected objects and optical densities are automatically measurable. This aspect will not be directly supported in the field trial versions, but such uses will be encouraged by both in-house and extramural users.

Initially, statistical analysis of the results of field trial will be only in its capacity as a grain counter.

Within the Image Processing Unit, it is planned to continue to use the grain counter as such, as well as to perform a complex set of quantitative analyses (including densitometry) on L1210 leukemia and its induced lymphocyte transformation.

Honors and Awards: None

Publications:

Glass, R. and Lemkin, P.: MAGTAP - A magtape utility program for the PDP-8. DECUS. In press.

Lemkin, P.: LIST - A batch processing utility for OS/8. DECUS. In press.

Lemkin, P.: UTIL2.SB - A modified UTILITY.SB for PS/8 (OS/8) FORTRAN II. DECUS. In press.

Lemkin, P.: MAGDMP - An OS/8 TC58 magtape file storage and retrieval system. DECUS. In press.

Glass, R. and Lemkin, P.: MAGTAP - A TC58 magtape handler for OS/8 FORTRAN II. DECUS. In press.

Lemkin, P.: An inter-PDP8 batch file transmission program. DECUS. In press.

Lemkin, P.: FAILSAFE - A disk/magtape utility for use with OS/8. DECUS. In press.

Lemkin, P.: OS/8 FORTRAN II logical arithmetic subroutines. DECUS. In press.

Lemkin, P.: Dicomed 31 image display device handler. DECUS. In press.

Lemkin, P.: An OS/8 FORTRAN II function to do BCD/decimal number conversion. DECUS. In press.

Lemkin, P.: Program to put a LINC8 block 0 bootstrap to bring in the OS/8 system disk. DECUS. In press.

Lemkin, P.: PT08/Datapoint 3300 PS/8 build programs. DECUS. In press.

Lemkin, P.: DATE - A FORTRAN II OS/8 subroutine to return the OS/8 date. DECUS. In press.

Lemkin, P.: Double precision arithmetic package for OS/8 FORTRAN II. DECUS. In press.

Lemkin, P.: GTCHR - An OS/8 FORTRAN II subroutine to analyze a character input stream from a SYS:DATA FILE. DECUS. In press.

Lemkin, P.: PS/8 EDIT modified for high speed PT08 and DC02G serial interface. DECUS. In press.

Lemkin, P.: DELETE - A program to delete illegal OS/8 files. DECUS. In press.

Lemkin, P.: SENDIT/SENDME - A PDP10 to PDP8 file transmission utility program. DECUS. In press.

Serial No. NCI - 6402
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 Through June 30, 1973

Project Title: Tumor Growth Kinetics and Chemotherapy

Previous Serial Number: NCI-6109

Principle Investigator: Stanley E. Shackney

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2
Professional: 1
Others: 1

Objectives:

1. To develop a model which will describe the growth of tumors in such a way that response to various chemotherapeutic agents can be simulated.

2. To apply the growth model to specific tumor systems.

3. To explore the pharmacodynamics of chemotherapeutic agents in specific tumor systems with the aid of the model.

4. To develop an approach to chemotherapy of tumors based on the expected interaction between tumor growth kinetics and drug pharmacodynamics.

Methods Employed:

Computer modelling techniques, *in vivo* and *in vitro* cell propagation techniques, radioautography with tritiated thymidine.

Major Findings:

1. Modelling Studies

a. Over the past year the portion of the model dealing with the simulation of PLM curve behavior was refined and completed. A general analysis of PLM curve, covering tissues ranging from rapidly growing mouse

tissues to slowly proliferating human cancers was done. Major findings include the following:

i. Clinical kinetic data can provide information regarding kinetic differences between normal and neoplastic tissues and among neoplastic populations when the data are analyzed for patterns of labelling intensity over the course of the cell cycle, rather than for phase durations.

ii. From the available data it appears that three distinct DNA synthesis patterns can be recognized. Normal squamous epithelia from a variety of sources and in different hosts exhibit a type II pattern with great regularity. Adult human leukemia also appear to exhibit this pattern. A variety of epidermoid carcinomas exhibit an intermediate pattern; experimental sarcomas and human breast cancer appear to exhibit a type I pattern.

iii. The apparent specificity of these patterns for given tissue types suggests that radioautographic methods can be used for the kinetic classification of clinically important normal tissues and various neoplastic populations. It would seem that with the appropriate analytic approach provided by the model, the time is ripe for extensive clinical kinetic studies in cancer patients.

The PLM curve simulation studies are described in two manuscripts which have been submitted for publication.

b. Over the past year, the model has been rewritten to accommodate the simulation of multiple populations simultaneously. This feature will be important for later explorations of differential effects of drugs on tumor vs. critical normal tissues, and for comparisons between the responses of primary and metastatic disease to drug therapy.

c. Over the past year the model has been expanded to permit the simulation of in vitro growth, and transplantation back and forth from in vivo to in vitro. Several more months of work remain before this part of the model is complete. This aspect of the model will prove to be of considerable importance in the later simulation of the kinetic interactions of drugs with growing cell populations, since the most precise quantitative information on drug kinetics has been collected in in vitro systems. In vitro simulations will also dovetail nicely with our current and projected experimental work (vide infra).

d. Over the past several months, work has begun on the simulation of kinetic parameters other than the PLM curve. These include DNA content distributions, long term tritiated thymidine infusions, labelling indices, grain count halving studies, and combined DNA content distribution - labelling studies.

The DNA content distribution is a parameter which appears to be a prime candidate as a rapid, accurate and repeatable clinical kinetic laboratory test for the initial kinetic classification of tumors and the subsequent

monitoring of drug response.

Simulations of long term tritiated thymidine infusions are currently under way. Preliminary studies suggest that information can be obtained from such studies which complements that of PLM curve studies, especially when the labelling rate of mitoses is compared with that of interphase cells. This work is still in an early stage, and is expected to continue through the coming year.

e. Work has begun on the modelling of drug characteristics. This is in a very early stage of development, and should come into focus later in the coming year, after the in vitro simulation package is completed.

2. Experimental studies

a. PLM curve studies

This past year, work was completed on the effects of counting threshold and emulsion exposure duration on the shape of the PLM curve. It was found that the shape of the PLM curve depended on these factors, suggesting that DNA synthesis is not restricted to a particular phase of the cycle, thus corroborating our modelling studies. A manuscript describing this work has been submitted for publication.

b. Bone marrow studies

This year, preliminary studies have been done on kinetic-cytoarchitectural correlations in mouse bone marrow. Mouse bone marrow appears to have a considerable degree of hitherto unsuspected cytoarchitectural organization. Radioautographs are currently being prepared which, it is hoped, will demonstrate the migration of label with time from the subendostial region to the granulocytes lining the sinusoids. A preliminary report of these findings is being prepared for publication.

This method shows considerable promise for the study of the type and degree of damage produced by various drugs on the hematopoetic system, and for the study of the kinetics of hematopoetic recovery.

c. The correlation of the DNA content distribution and PLM curve behavior in late stages of growth.

Preliminary studies have suggested that the PLM curve undergoes pronounced changes in late stages of growth in our in vitro sarcoma 180 system. More rigorous studies of the PLM curve in late stages of growth are currently in progress, and concurrent DNA content distributions are being obtained at different stages of growth.

Significance to Biomedical Research and the Program of the Institute

Hopefully, a better understanding of the growth characteristics of tumors, and the way in which growth affects response to chemotherapeutic agents, will lead to a rational, systematic approach to the chemotherapy of tumors,

both experimentally and clinically, and provide the means for translating experimental data obtained in animals into clinically useful information.

Proposed course

1. Modelling studies

Modelling studies are expected to continue on several fronts:

a. Exploration of kinetic parameters other than the PLM curve,

b. The simulation of *in vitro* growth,

c. The simulation of drug characteristics, and

d. The simulation of drug-population interactions.

2. Experimental studies.

a. PLM curve studies

At present, we are collecting radioautographic data in late stages of growth. This work should be completed over the summer of 1973. These data, together with the data already obtained in log phase growth, and DNA content distribution data at difference stages of growth should permit comprehensive modelling of our *in vitro* system.

b. Bone marrow studies

Studies on kinetic-cytoarchitectural studies of the unperturbed normal mouse bone marrow should be completed by the summer of 1973. The effects of drugs on mouse bone marrow will subsequently be studied.

c. DNA content distributions

A study of DNA content distributions, during normal growth and following perturbation by drugs will be undertaken during the coming year. As of now, it is anticipated that we will have access to the cytoflourograph in Dr. V. Bono's laboratory, and in addition, limited access to the Los Alamos machine being installed in Dr. C. Herman's laboratory.

d. Drug studies

During the coming year it is anticipated that studies will begin on drug perturbation effects. The main emphasis of these studies will be to characterize the type and degree of sublethal damage induced in surviving cells and its effects on the subsequent kinetic behavior and drug responsiveness of these cells.

3. Clinical studies

Our current efforts have brought us to the point where we believe that clinical data can be collected and interpreted in a meaningful way for

therapeutic decision making. Plans are being made for the establishment of protocols for kinetic studies on cancer patients at the Clinical Center.

Preliminary discussions are being held with Dr. Marc Straus at the VA hospital regarding the collection of kinetic data in patients with lung cancer. An advisory, and possibly a collaborative role is envisioned for us in these studies.

SUMMARY REPORT

DRUG RESEARCH AND DEVELOPMENT (DR&D)

DIVISION OF CANCER TREATMENT (DCT)

Drug Research and Development has as its primary task the search for and development of new and potentially more effective antitumor agents, as well as the development of better methods of drug selection and prediction of optimal therapy with new and old drugs and drugs combined with other treatment modalities. With respect to the Division of Cancer Treatment (DCT) Linear Array, the organization is responsible for all preclinical work in the development of drugs except that concerned with toxicology and pharmacology. It also includes information and communication functions in support of other portions of the Division.

The DR&D program includes both in-house laboratory operations and contracts with pharmaceutical and chemical companies, research institutes and academic institutions, with the major portion of the funding directed toward the contract-supported activities. The program depends heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts. The program activities cover the following specific areas:

1. Synthesis of new chemical agents.
2. Procurement and resynthesis of bulk chemicals and drugs for preclinical and clinical evaluation.
3. Development of new agents from fermentation, plant and other natural products.
4. Development and production of clinical dose forms for clinical trial.
5. Quality control for bulk and formulated drugs.
6. Primary screening and evaluation of new materials.
7. Bioassays, both in vivo and in vitro, to monitor the purification of fermentation and other natural products.
8. Study of optimal drug routes and regimens, including combination therapy and other means for improving drug effectiveness.
9. Development of new and improved laboratory methods for discovering, evaluating and utilizing potential antitumor agents.
10. Investigation of mechanisms of action and resistance of drugs of clinical interest.
11. Animal procurement, distribution and disease control.
12. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies.
13. Development and operation of data processing systems for both biological and chemical information.
14. Search, retrieval and dissemination of pertinent information from the world's literature on drugs and other aspects of cancer therapy.
15. Publication of the abstract journal, Cancer Chemotherapy Abstracts.
16. Publication of the scientific journal, Cancer Chemotherapy Reports.

During Calendar Year 1972, materials for screening were obtained through a variety of mechanisms, including direct purchase, synthesis contracts, natural

product development contracts and voluntary submissions. To the limits of the state of the art, new compounds are synthesized on the basis of biochemical, chemical, pharmacological and other rationale. DR&D contractors synthesized 1,125 new structures in 1972. Their effort was distributed into many chemical areas, as shown in Table 1. Since we cannot as yet predict what type of chemical structure may demonstrate activity, we continue to obtain a wide variety of structures from as many sources as possible. As a part of the overall expansion of the National Cancer Program, an increased rate of acquisition was accomplished in 1972, as shown in Table 2.

TABLE 1

Contract Synthesis Program

Distribution of Effort

Areas	Effort %
Nucleosides & Purine Antagonists	16
Alkaloids	13
Antibiotics	13
Nitrogen Heterocycles	9
Folic Acid Antagonists	9
Alkylating Agents	7
DNA and Receptor Site Oriented Agents	5
Molecular Control - Polymerase Inhibitors	5
Oxygen and Sulfur Heterocycles	4
CNS - Brain Antitumor Agents	4
Coordination Compounds	3
Imidazoles and Pyrimidines	2
Quinones	2
Miscellaneous	8
Total	100

TABLE 2

Input and Output of Drug Development Program

	Calendar Year						
Materials Submitted	1966	1967	1968	1969	1970	1971	1972
Synthetic & crystalline natural products	8,008	7,413	10,915	9,820	8,521	10,151	15,770
Crude fermentation products	4,284	4,982	4,256	2,184	2,172	1,215	2,712
Crude plant products } 3,620		6,253	4,245	5,109	3,499	3,015	10,434
Animal products		446	641	757	587	1,203	1,669
Total	15,912	19,094	20,057	17,870	14,779	15,584	30,585
IND applications filed	3	4	5	5	6	8	5

Levels of biological screening during 1972 demonstrated an increased effort in parallel with the increased input. A comparison of screening tests for the past five years is shown in Table 3.

TABLE 3

Screening Tests

	Calendar Year				
	1968	1969	1970	1971	1972
L1210 Lymphoid Leukemia	107,445	80,545	79,892	102,424	103,681
P388 Lymphocytic Leukemia	14,903	43,223	42,270	53,449	180,027
Walker Carcinosarcoma 256	44,563	20,328	10,247	4,368	2,770
B16 Melanoma	286	1,240	1,342	1,423	6,028
Lewis Lung Carcinoma	244	925	3,033	2,803	4,141
Other Tumors	774	426	504	1,780	6,458
Total In vivo	168,215	146,687	137,288	166,247	303,105

As a result of the screening and related drug development efforts, nine materials passed Decision Network 2A in 1972. That is, they demonstrated sufficient biological activity and chemical or other uniqueness to warrant the effort in dose formulation development, etc., leading to preclinical toxicology and clinical trial. These materials are listed in Table 4.

TABLE 4

Compounds Passing Decision Network 2A

NSC No.	Compound Name	Source
104801	Cytembena	Synthetic
139490	Homofolic acid, 5,6,7,8-tetrahydro-5-methyl-	Synthetic
141633	Homoharringtonine	Plant
142982	Hycanthone monomethanesulfonate	Synthetic
143057	Quinaldine, 4,4'-(hexamethylenediimino)bis[6-amino-, dihydrochloride	Synthetic
143414	Rifamycin, 4-benzyl-2,6-dimethyl-	Synthetic
153858	Maytansine	Plant
163062	Triptolide	Plant
163063	Tripdiolide	Plant

The coordinated preclinical drug development work in 1972 resulted in five IND applications being filed, signifying the initiation of clinical trials. These materials are listed in Table 5.

TABLE 5

IND'S Filed

NSC No.	Compound Name	Source
58514	Chromomycin A	Fermentation
68075	Thalicarpine	Plant
122819	Epipodophyllotoxin, 4'-demethyl-, 9-(4,6-O-2-thenylidene-β-D-glucopyranoside)	Synthetic*
141540	Epipodophyllotoxin, 4'-demethyl-, 9-(4,6-O-ethylidene-β-D-glucopyranoside)	Synthetic*
404241	Adenine, 9-β-D-arabinofuranosyl-	Synthetic

* Synthetic modification of plant materials

Because of the magnitude and diversity of research and development efforts within DR&D, only a few selected aspects may be described in this brief summary report.

One of the highlights of the past year's activities relates to the evaluation of the so-called "mini-screen." Increasingly, synthetic compounds are being submitted in small quantities, and in vivo testing is, therefore, becoming less feasible using the normal protocols. The exploratory scheme that was investigated made several changes in the L1210 procedure, among them being: (a) use of three mice per test group rather than six, (b) use of one treatment schedule instead of two, (c) use of more than the usual three dosage levels, and (d) adjustment of dosage level and protocol in accordance with the quantity of drug available. Experiments with a series of known drugs demonstrated that, at least with the highly reproducible L1210 system, known active agents could be selected if submitted in quantities of 20 mg. or more. Through appropriate modifications of this experimental screening procedure, it is possible to carry out the primary screening of new agents not otherwise feasible and also to do so at a considerable saving in dollars. Consideration is now being given to the conversion of the entire L1210 primary screen to this type of procedure. It should be recognized that such a procedure is applicable only to a highly reproducible system. Furthermore, it is likely that more false positives will result from the preliminary screen and further testing will be needed to eliminate them. However, overall savings in dollars, animals and quantities of drug are well worth the small additional testing effort.

The screening development described above, which opens the possibility for screening thousands of compounds that could not otherwise be tested, combined with the publicity received in relation to the National Cancer Program and the intensive efforts to tap new sources of compounds in this country and abroad, results in a potential influx of more chemical compounds than would be feasible to test even under the simplified protocols. Serious consideration is, therefore, being given to devising methods that might be utilized for selecting compounds to be tested or at least to developing a system of priorities for the testing of large numbers of compounds. It is relatively simple to identify a few classes of compounds that one would certainly wish to test, e.g., analogs of clinically effective compounds, but these represent a small percentage of the total number of compounds offered for testing. It is also possible to identify

a few selected classes of chemical structures that would not be of interest for testing, e.g., aliphatic hydrocarbons and a few classes that have been tested extensively and found to be devoid of activity. Unfortunately, the vast majority of compounds fall into less definable categories somewhere between the first two. Efforts are, therefore, being made to obtain the best advice possible to attempt to classify compounds in some manner that will enable us to set the priorities that may be necessary. The problem is not a simple one and the solution may require considerable time and careful thought. We constantly discover new chemical structures with biological activity and it is, therefore, extremely dangerous to arbitrarily eliminate certain classes of compounds from consideration from testing unless truly adequate evaluation of the class has been carried out. However, the influx of compounds may be so great that this will be necessary.

The organizational staffing of Drug Research and Development has remained very stable over the years. The need for a more organized experimental effort in biology and biochemistry in our laboratories in Building 37 has been recognized, however, and late in FY 1973 the Laboratory of Experimental Chemotherapy was officially created. It is hoped that this laboratory may be fully staffed within the next few years and thus be able to actively carry out exploratory studies in areas of vital interest to the DCT program.

Staff and Organization

The DR&D laboratory and contract activities during FY 1973 were carried out by a full- and part-time staff of 118: 20 senior professionals (GS-13 or higher), 27 junior professionals, 12 guest workers and fellows, 17 laboratory technicians and support personnel, and 42 clerical and secretarial personnel. The present organization is outlined below:

Drug Research and Development

Associate Scientific Director for Drug Research and Development -
Saul A. Schepartz

Associate Chief for Laboratory Research - Abraham Goldin
Mammalian Genetics and Animal Production Section - Samuel M. Poiley, Head

Drug Development Branch - Harry B. Wood, Jr., Chief

Chemical and Drug Procurement Section - Robert R. Engle, Head
Chemical and Drug Records Section - Robert B. Ing, Head
Clinical Drug Distribution Section - J. Paul Davignon, Head
Natural Products Section - Jonathan L. Hartwell, Head

Drug Evaluation Branch - John M. Venditti, Chief

Biochemistry Section - Florence R. White, Head
Immunity & Immune Response Modifiers Section
Screening Section - Betty J. Abbott, Head

Program Analysis Branch - Barbara R. Murray, Chief

Literature Research Section - Dorothy Algire, Head
Publications Section - Pamela Hutchins, Head
Section on Data Processing and Computer Systems Development - Sidney Richman, Head
Scientific Records Section - George F. Hazard, Acting Head

Laboratory of Experimental Chemotherapy

The reports of each of the Branches and Sections which follow will present in great detail the accomplishments and future directions in each area.

Serial No. NCI 3575

1. Office of Associate Scientific Director, Drug Research and Development, Division of Cancer Treatment, NCI
2. --
3. NCI-Building 37 and Microbiological Associates, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Chemotherapy research including fundamental biological and biochemical investigation of antitumor agents

Previous Serial Number: Same

Principal Investigator: Abraham Goldin

Co-Principal Investigators: A. W. Schrecker, R. K. Johnson, J. M. Venditti, O.C. Yoder

Other Investigators: D. P. Houchens, V. Subba Reddy, A. Miyakawa, A. A. Ovejera

Cooperating Units: G. Cudkowicz, State University of New York at Buffalo; J. M. Venditti and Staff, Drug Evaluation Branch, DCT, NCI; H. B. Wood, Jr., R. R. Engle, J. Driscoll and J. Hartwell, Drug Development Branch, DCT, NCI; J.A.R. Mead, Experimental Therapeutics, DCT, NCI; R. Gallo, Human Tumor Cell Biology Branch, DCT, NCI; D. Cooney and Staff, Laboratory of Toxicology, DCT, NCI; N. Mantel, Biometry Branch, NCI; M. Sporn, Carcinogenesis, NCI; T. Borsos, B. Zbar and Staff, Biology Branch, NCI; P. Carbone, Medicine Branch, NCI; Y. Ikawa, Viral Leukemia and Lymphoma Branch, NCI; H. E. Skipper, F. M. Schabel, Jr., and Staff, Southern Research Institute; I. Kline, S. Vadlamudi, and M. Kende, Microbiological Associates, Inc.; I. Wodinsky, A. D. Little Company; D. Hutchison, Sloan-Kettering Institute; E. Frei III, Children's Cancer Research Foundation, Boston; A. DiMarco, Farmitalia, Milan, Italy; E. Bonmassar, University of Milan and University of Perugia, Italy; A. Nicolin, University of Milan, Italy; S. Garattini, Instituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy; C. P. Li, Bureau of Biologics, FDA.

Man Years
Total: 9-11/12
Professional: 6-1/3
Others: 3-7/12 (plus animal care support from Mammalian Genetics and Animal Production Section, DR&D)

Project Description:

Objectives:

To conduct fundamental investigations of potential and established clinical cancer chemotherapeutic agents; to obtain biological and biochemical knowledge concerning chemical agents capable of affecting malignant growth; to determine means for improvement of therapy with existing drugs; to investigate the influence of structural modification of drugs on the mechanism of antitumor action; to provide basic information of pertinence to the clinical use of such agents; to study specific problems encountered during the use of these agents in the clinic.

Specific objectives include:

I. Investigations of primary and secondary screening procedures and methodology employed in drug evaluation.

   A. Investigation of characteristics of ongoing screens and drug evaluation systems and of new test systems which may broaden the base of predictability of drugs for clinical activity. Emphasis is placed on predictability systems for clinically useful compounds which were not predicted by the standard screen and systems for the selection of new and novel structures which may have potential in clinical chemotherapy.

II. Investigation of the influence of drugs on tumor cells.

   A. Detailed investigation of mode of action of drugs including purine and pyrimidine antagonists, folic acid antagonists, alkylating agents, natural products, etc.

      1. Study of the influence of drug on the mitotic cycle.

      2. Study of the lethal action of tumor cells.

      3. Investigation of problems related to drug resistance.

      4. The influence of chemical agents on metastasis and tumor cell sequestration including intracranial involvement.

      5. Investigation of the sites of action of drugs.

      6. Study of the influence of drugs on the hematopoietic and tissue components of the host in relation to antitumor activity.

      7. Inhibition studies of RNA-dependent DNA polymerase.

   B. Investigation of factors which may influence drug action in relation to improvement of therapy.

III. Study of the influence of drugs on tumor-host interactions.

A. Investigation of the basis for drug specificity.

1. Exploration of the influence of novel schedules.

Investigation of the underlying basis for drug scheduling and combination chemotherapy.

2. Detailed studies in inhibition analysis.

a. Determination of metabolite-antimetabolite interrelationships for folic acid congeners, purine antagonists, etc.

b. Investigation of the inhibition of de novo synthesis, lethal synthesis, feedback inhibition, etc.

c. Investigation of collateral sensitivity as a phenomenon which may lead to improved therapy.

B. Exploration of means for augmentation of the host contribution to chemotherapy.

C. Chemotherapeutic investigations on tumor cell kinetics with a view to the total eradication of the tumor cell population in the host.

IV. Antiviral chemotherapy.

A. Study of the action of drugs on virus-induced tumors.

V. Exploration of new and potentially useful biological and biochemical methodology for prediction, evaluation and improvement of drug therapy.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute.

In addition to the laboratories in our building, the Kenwood Laboratories of Microbiological Associates staffed by Microbiological Associates professional and technical personnel serve as a collaborative laboratory resource for the research staff of this program. The chemotherapy research program collaborates closely with the ongoing programs in drug evaluation, drug development, immunology, organic chemistry and pharmacology. The program serves as a focal point for the interdigitation of chemotherapy research including the DR&D screening, developmental and basic chemotherapeutic investigations with collaborating DR&D contractors, and clinical programs of NCI and other institutions. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes, both in the United States and abroad in areas pertinent to chemotherapy.

Methods Employed: Standard laboratory procedures were employed.

International Activities Including Surveillance for New Agents

An important function of this office involves the maintenance of close relationships with world-wide cancer chemotherapy programs with a view to (a) information exchange pertaining to latest scientific developments in chemotherapy, (b) for the opening of new avenues of procurement of synthetic agents and natural products for screening and (c) for the identification of new compounds of potential interest for chemotherapy.

In the above, close liaison is being maintained with investigators in leading institutions, including universities, medical schools and pharmaceutical houses abroad. Active contact continues to be maintained through the Chemotherapy Committee of the International Union Contra Cancrum, the International Agency for Research in Cancer of the World Health Organization, Lyon, France and the European Organization for Research and Treatment of Cancer (EORTC). The Division of Cancer Treatment Offices which have been established at the Cancer Institute in Tokyo, Japan and at the Institut Jules Bordet, Brussels, Belgium provide a mechanism for close collaboration encompassing both the preclinical and clinical cancer chemotherapy programs of Asia and Europe with that of the National Cancer Institute. These offices have been functioning successfully to gather and exchange preclinical and clinical scientific knowledge in the further development of cancer chemotherapy. They are increasing the input into the chemotherapy screening program at the National Cancer Institute and fostering collaboration in the study of new anticancer drugs. In addition to close contact with universities, medical schools, chemical industry and pharmaceutical houses in the various countries, the offices are providing a means for the continuous review, abstracting, and translating of significant foreign reports.

In order to further progress in the European area Dr. Omar Yoder has been designated to serve as an NCI Division of Cancer Treatment officer at the European office in order to further the overall collaboration in Europe. He has made significant contacts with various pharmaceutical houses, chemical industry and universities in the acquisition of new compounds for screening and in the overall exchange of preclinical and clinical information. In addition to his activities at the Jules Bordet Institut he has been making visits to important institutions in the various countries of Europe, particularly those involved in the activities of the European Organization for Research and Treatment of Cancer (EORTC). The program at the Jules Bordet Institut has been enlarged to encompass a screening program for new antitumor agents. The materials tested in the screening program are collected by the DCT office in Europe. A screening program has also been instituted at the Mario Negri Institute in Milan for the testing of compounds collected primarily in southern Europe.

A screening program has also been instituted at the Cancer Institute in Tokyo as part of our DCT collaborative program for the testing of compounds collected in Japan. The new building of the National Cancer Institute in Japan which will serve as the "Anticancer Drug Evaluation Center" is nearing completion and it is estimated that the building may be available for occupancy in May 1973. The DCT offices in both Brussels and Tokyo have publicized the DCT program of the National Cancer Institute in Bethesda extensively in the

various countries of Europe and Asia.

In relation to the U.S.-Russian cooperative medical agreement contacts have been broadened with Soviet cancer chemotherapists and plans have been made for a preclinical chemotherapy team to visit preclinical facilities in the Soviet Union for purposes of broadening and facilitating exchange of information, drugs and data and for the expediting of the overall collaborative effort. The preclinical relationship with the Hungarian Cancer Institute is also under discussion in this regard.

Visits were made by A. Goldin to the following:

1. A visit was made by A. Goldin and S. Carter to the DCT Liaison Office in Japan to meet with various Japanese investigators and to learn about the status of new chemotherapeutic agents and drug trials in Japan. A meeting was held with Professor Tomizo Yoshida and Professor Yoshio Sakurai and their staff concerning the functions of the new DCT office and future plans for the new building that is being constructed by the institute.

In Osaka a visit was made to the Center for Adult Diseases, and discussion was held with clinical investigators at that institution. A visit was also made to the laboratories of the Takeda Chemical Industries, Ltd. to discuss further cooperation in the submittal of new compounds for screening and to review their ongoing research program.

A visit was made to the Aichi Cancer Center in Nagoya in the company of Dr. Akira Hoshino and various members of the staff. Dr. Hoshino, who is a leading young preclinical and clinical investigator in Japan, is planning to join the Tokyo DCT Office in April.

In Tokyo a visit was made to Dr. Kimura and his staff at the Tokyo Cancer Center for review and discussion of ongoing experimental and clinical programs. A two-day U.S.-Japan Joint Seminar on Anticancer Drug Evaluation was held during this visit at the Tokyo Cancer Institute. Specific attention was focused on new antitumor agents emanating from Japan.

In relation to this program a visit was made by Drs. Tsukagoshi and Hoshino to our DCT program in Bethesda for approximately one month to permit updating for the Japanese investigators in all aspects of our program including drug evaluation and development and the clinical programs.

2. A meeting was attended of the Preclinical and Clinical Pharmacology Cooperative Group of the European Organization for Research on Treatment of Cancer (EORTC) at the Jules Bordet Institut where ongoing preclinical programs in Europe were discussed. This visit also provided an opportunity to participate in the Annual Assembly of the EORTC where there was a symposium on "Aspects of Immunology in Cancer". There is a strong interest in the immunological approach to therapy in the EORTC and in the relation of this approach to chemotherapy.

3. Attendance at an international symposium on "Chemotherapy of Cancer Dissemination and Metastasis" at the Mario Negri Institute in Milan. This symposium was sponsored by the International Union Against Cancer. At this session A. Goldin presented a paper on "Effects of Drugs on Disseminated Tumor", chaired a session on "Effects of Drugs on Host-Tumor Relationship" and participated in a round-table session on "Critical Evaluation of the Symposium". This symposium was particularly useful for reviewing the international effort in preclinical chemotherapy of cancer dissemination and metastasis.

4. Attendance at the International Cancer Conference and the 8th International Pigment Cell Conference held under the auspices of the International Union Against Cancer and the Australian Cancer Society in Sydney, Australia. Sessions were attended on leukemia and melanoma but major participation was at the leukemia sessions where there was considerable emphasis on virologic and immunologic aspects of leukemia. The meetings served an excellent function in making Australia, isolated geographically, a focal point for gathering of medical scientists and latest discussion in cancer research.

5. Served as one of the organizers and attended a U.S.-Australian Conference on Current Cancer Research which was held at the Royal Children's Hospital in Melbourne, Australia. This meeting was organized under the agreement for scientific interchange and collaboration between the governments of Australia and the United States. The sessions were devoted to virological aspects of cancer, biochemical and biological aspects of cancer, immunologic aspects of human cancer, and biological and immunological approaches to therapy and the chemotherapy of cancer. In addition to presenting a paper plus participation in overall discussion the sessions provided an excellent opportunity for review of latest developments in Australia in immunology and virology in relation to chemotherapy, areas in which the Australians have great expertise. A meeting was held in Melbourne with members of the Commonwealth Scientific and Industrial Research Organization concerning the submittal of natural products and synthetic compounds from Australia to our screening program.

6. A visit was made to Dr. Bruce F. Cain and his colleagues of the Auckland Division of the Cancer Society of New Zealand for discussion of compounds he has submitted for screening. Dr. Cain has an active research group encompassing organic synthesis, screening and evaluation of drugs, and several of his compounds have proven to be of considerable interest to our program. This visit provided an opportunity for the working out of specific logistic problems in our collaboration, dictated by the geographic isolation. Several aspects of the collaborative program with Dr. Cain have been implemented as the result of this visit.

7. Participated in a course in clinical pharmacology held under the auspices of the European Organization for Research on Treatment of Cancer (EORTC) with the participation of the International Agency for Research on Cancer, Lyon. The course was held at the Institut Jules Bordet in Brussels and the proceedings were published in a book "The Design of Clinical Trials in Cancer Therapy". Drs. S. Carter and A. Goldin presented

a series of lectures at this course. It is considered that this type of program is of considerable importance in terms of our collaborative interest.

8. Attended a Symposium on the Mechanism of Action of Cytostatic Agents at the Hungarian Academy of Sciences in Budapest. In addition to participation at the meeting a paper was presented (by A. Goldin and J. M. Venditti) on the subject of "Schedule Dependency of Antitumor Agents". An opportunity was provided on this trip to visit the National Cancer Institute in Budapest of which Professor S. Eckhardt, who worked in our laboratory as an Eleanor Roosevelt Fellow, is now the Director. The program at this Institute is expanding both in the preclinical and clinical areas with the construction of new buildings. Collaboration with the Hungarian scientists has been good and it is hoped that as a result of this visit there may be expansion of these relationships in both preclinical and clinical areas.

The following talks describing various aspects and results of our program were given abroad during the year by A. Goldin.

1. Current Status of the Preclinical Drug Evaluation Program with Reference to New Drugs of Interest. Presented at U.S.-Japan Joint Seminar on Anticancer Drug Evaluation at the National Cancer Institute in Tokyo.

2. Closing Address. At U.S.-Japan Joint Seminar on Anticancer Drug Evaluation at the National Cancer Institute in Tokyo.

3. Effects of Drugs on Disseminated Tumor. Presented at International Symposium on "Chemotherapy of Cancer Dissemination and Metastasis" at the Mario Negri Institute in Milan.

4. Participant in round-table discussion "Critical Evaluation of the Symposium". At the International Symposium on Chemotherapy of Cancer Dissemination and Metastasis, Mario Negri Institute, Milan.

5. Immuno- and Chemotherapeutic Approaches in Preclinical Chemotherapy. Presented at U.S.-Australian Conference on Current Cancer Research in Melbourne, Australia.

6. Presented a series of four lectures at a course in Clinical Pharmacology on the Design of Clinical Trials in Cancer Therapy at the Jules Bordet Institut in Brussels as follows:

a. Animal Tumor Model - Introduction to the Rationale of Screening Tests.

b. Importance of Dose Schedule and Route of Administration.

c. Experimental Screening Procedure and Clinical Predictability Value.

d. Quantitative Comparison of Toxicity in Animals and in Man.

7. Schedule Dependent Drug Effect. (A. Goldin and J. M. Venditti). Presented at Symposium on Mechanism of Action of Cytotoxic Agents at Hungarian Academy of Sciences, Budapest.

Additional talks presented in the United States include:

1. Preclinical Characterization of Antitumor Agents. Presented at the Midwest Regional American Chemical Society Meeting at the University of Missouri, Columbia, Missouri.

2. Preclinical Antitumor Drug Evaluation as Illustrated by the Folic Acid Antagonists. Presented at the N.E. Section of the American Chemical Society Meeting in Boston, Massachusetts.

3. Rationale of Combination Chemotherapy Based on Preclinical Experiments. Presented at the International Workshop for Therapy of Lung Cancer at the Airlie House, Warrenton, Virginia.

A Review of Antineoplastic Substances from the Sea. (C. P. Li, A. Goldin and J. L. Hartwell).

Because of the interest in our chemotherapy program in the sea as a potential source of chemotherapeutic agents, an extensive review was conducted of the status of antitumor agents from the sea derived from both animals and plants including the methods of preparation, methods of testing, the biochemical characteristics and the possible mode of action. This review has been completed and will be published in Cancer Chemotherapy Reports. The article shows that a number of authors have demonstrated activity against experimental tumors in marine materials from various localities, with differing methods and at different times. Although in most cases the antitumor activity has been only marginal, the presence of antitumor agents in the sea has been established. To date most of the antitumor substances from marine origin are relatively impure demonstrating a number of biological activities some of which may be of therapeutic value while others are harmful to the host. It would appear that the sea remains as a largely untapped resource in the search for new antitumor agents. The broad range of marine animals and plants from which biologically active products and some antitumor substances have been obtained suggests that continuing effort be made in the screening evaluation development, identification and determination of the mechanism of action of antitumor agents from the sea.

A Review of Combination Chemotherapy. (A. Goldin, J. M. Venditti and N. Mantel).

In view of the demonstrated improved chemotherapeutic results obtained with combinations of drugs in the clinic and the resultant strong clinical interest a review of combination chemotherapy in experimental tumor systems was undertaken. A review article "Combination Chemotherapy: Basic Considerations" was written by A. Goldin, J. M. Venditti and N. Mantel for "A Handbook of Experimental Pharmacology - Antineoplastic and Immunosuppressive Agents". In the chapter there is focus on principles of combination drug evaluation.

Examples are given of (a) therapeutic synergism resulting from decreased host toxicity without concomitant decrease in effectiveness against the tumor, (b) therapeutic synergism resulting from selective increase in antitumor toxicity using combinations of individually active agents, (c) attempts to improve therapeutic response by altering the concentration and duration (c X t) of an antitumor agent. Subjects taken under consideration and illustrated include schedule dependency in combination chemotherapy, sequential combination chemotherapy and the dosage ratio in drug combinations. There is a discussion of the biochemical rationale in the choice of drug combinations. Additional subjects include the use of drug combinations to overcome resistance to treatment, combination chemotherapy of meningeal leukemia, combination chemotherapy of spontaneous AKR lymphoma, chemotherapy plus immunotherapy and surgery and chemotherapy (surgical adjuvant therapy).

In general, combination chemotherapy studies in antitumor systems have been conducted with known antitumor agents. It is stressed that with increasing knowledge it may be possible to select antitumor agents for combination chemotherapy on a wholly rational basis and several successful attempts in this direction are cited. The studies stress further the great need for close collaboration between preclinical and clinical investigators in the studies of combination chemotherapy.

Major Findings:

I. The Relationship of Structure to Antitumor Activity

The program relating structure to antitumor activity has been continued in collaboration with Drs. Wood, Engle, Driscoll, Mead, Venditti, Johnson and Sandberg. The structure-activity studies of the methanesulfonates has now been completed and will be published in Cancer Chemotherapy Reports. The report on benzoquinone derivatives is nearing completion and in this extensive series the structure-activity relationships are being analyzed by Dr. Driscoll employing computer methodology.

Studies of the relation of structure to antifolate activity have continued. Additional new structures have been analyzed for inhibition of dihydrofolate reductase and antitumor activity against advanced leukemia L1210. A start has been made on structure-activity analyses of adriamycin and daunomycin derivatives and also for derivatives of actinomycin D. There is an ongoing program of synthesis in the area of structure-activity analyses under the direction of the Drug Development Branch.

II. Mechanism of Action of Antitumor Agents

A. Reverse Transcriptase Inhibitors. (A. W. Schrecker, R. C. Gallo, R.G. Smith and M. B. Sporn)

In attempts to explain the known selective antileukemic effect of cytosine arabinoside (ara-C), the inhibition of several DNA polymerases by its 5'-triphosphate (ara-CTP), its main intracellular metabolite, was investigated. This inhibition is known to be competitive with the DNA precursor dCTP and has been shown to be

responsible for its cytotoxic effect. DNA polymerases purified for this study by ion-exchange chromatography included the reverse transcriptase from Rauscher leukemia virus and the two main cellular DNA polymerases from cultured human normal and leukemic lymphocytes, namely, the high-molecular weight DNA polymerase I (presumably the replicative enzyme) and the low-molecular weight DNA polymerase II (presumably a repair enzyme). From the enzyme kinetic data obtained, the Michaelis-Menten constant (K_m) for dCTP and the inhibition constant (K_i) for ara-CTP were determined by a computer program. The RNA-directed DNA synthesis by the viral enzyme (with oligo dC. poly rI as the synthetic template) was inhibited for a far greater extent than that directed by activated salmon sperm DNA. Human lymphocyte DNA polymerase I (DNA template) was inhibited to about the same extent as the RNA-directed synthesis of the viral enzyme and more strongly than polymerase II. Inhibition of the enzymes from leukemic lymphocytes appeared somewhat greater, but not significantly so, than that of the enzymes from normal lymphocytes. In a preliminary experiment, the reverse transcriptase purified from the lymphocytes of a patient with acute lymphocytic leukemia was inhibited to about the same extent as the cellular polymerase I when DNA was the template.

Continuing the investigation of synthetic thymidylate derivatives with reactive substituents on the phosphate ester or deoxyribose moieties, it was found that one of the compounds (bromoacetamidotetrathymidylate) at an 0.5 mM concentration inhibited cellular DNA polymerase I and the reverse transcriptases from Rauscher leukemia virus and from a leukemic patient more than 95%, when DNA was the template, but caused only minor or no inhibition of the cellular polymerase II or the reverse transcriptase purified from Mason-Pfizer monkey mammary tumor virus. Compounds of this type could be useful to differentiate between reverse transcriptase and normal cellular enzymes.

B. Antiviral and Antineoplastic Properties of a Rifamycin Analog. (M. Kende, T. Soares and A. Goldin)

It has been observed that diphenylhydrazone 3-formyl rifamycin SV is capable of inhibiting reverse transcriptase activity of Moloney sarcoma virus *in vitro*. In the current studies it was observed that focus formation and reverse transcriptase activity were inhibited on pre-incubation of Moloney sarcoma virus with the drug but that no inhibition occurred when the drug was added subsequent to absorption of the Moloney sarcoma virus. Pre-incubation of the Moloney sarcoma virus with drug reduced the tumor incidence *in vivo* whereas injection of the drug following infection of mice with the Moloney sarcoma virus failed to reduce the incidence of tumor induction. Thus it would appear that pre-incubation of Moloney sarcoma virus with diphenylhydrazone 3-formyl rifamycin SV inhibits the reverse transcriptase of Moloney sarcoma virus and thus decreases the transformation of cells by the virus *in vivo*. Apparently the anti-viral and antineoplastic activity of the drug depend on its presence prior to infection.

C. Studies of the Interaction Between O-2,2'-Cyclocytidine (NSC-145668) and Arabinosylcytosine (NSC-63878). (R. Johnson, J.A.R. Mead and A. Goldin).

Recent evidence from several laboratories has suggested that cyclocytidine may inhibit deoxycytidine deaminase, the enzyme responsible for the rapid inactivation of ara-C.

We have evaluated the lethality in normal mice of simultaneous chronic administration of ara-C and cyclocytidine. The LD_{50} of ara-C was progressively reduced with increasing, non-toxic levels of cyclocytidine. This enhancement of toxicity could be due to either inhibition of the detoxification of ara-C or to enzymatic hydrolysis of cyclocytidine to ara-C to an extent where blood levels of ara-C derived from both treatments reached toxic levels.

Double-labelled studies with ^{3}H-ara-C and ^{14}-C-cyclocytidine are being performed to determine the relative contribution of these metabolic pathways to the interaction of the two drugs. These studies should indicate whether the suspected inhibition of deoxycytidine deaminase by cyclocytidine may be put to clinical use in prolonging effective blood levels of ara-C.

Therapeutic trials of the combination in tumor-bearing mice will be performed to show whether a therapeutic advantage can be obtained over single drug therapy with these two compounds.

III. Drug Evaluation Systems

A. Leukemia L1210 Mini Screen

Because of the availability of highly sophisticated instrumentation for the determination of chemical structure, chemists are capable of determining the structure of newly synthesized compounds with minimum quantities of material. As a result there are many new compounds of potentially interesting structure available in only short supply. In order to be able to examine these compounds for antitumor activity a screening system was devised for the purpose of testing small amounts of material (as low as 3-10 mg) for chemotherapeutic activity. A series of studies has now been completed by the Drug Evaluation Branch in two contract laboratories and the results indicate that the mini screen was highly successful in signalling activity for small amounts of compound. The schema is currently being employed on a wider scale for the signalling of antitumor activity to serve as a guide for detection of compounds that should be synthesized in larger quantity for further testing.

B. Comparison of Chemotherapy of AKR Lymphoma and Human Neoplastic Disease. (E. Frei III, F. M. Schabel and A. Goldin)

An extensive amount of quantitative data have been obtained with respect to the chemotherapy of spontaneous AKR lymphoma and also with first

generation AKR lymphoma. Since a great deal of clinical data is also available it was considered desirable to attempt correlations of antitumor agent responsiveness in the spontaneous AKR system and in appropriate human tumors. This study was undertaken by a subcommittee of the Ad Hoc Study Group chaired by C. Gordon Zubrod. The analysis to date indicates the following:

The best correlation between the chemotherapeutic results with spontaneous AKR lymphoma and specific disease categories in man appears to be with non-Hodgkin's lymphoma and with myeloma. It is suggested that it will be important to examine the various subcategories of non-Hodgkin's lymphoma with respect to correlation with the AKR spontaneous system. A summary ranking of the activity of agents in hematologic malignancies appears to correlate reasonably well with the spontaneous AKR ranking.

There does not appear to be any advantage in employing the spontaneous AKR system for primary screening as compared with the leukemia L1210 system, since there was good correspondence for the clinically active drugs tested in both systems. The first generation AKR leukemia gave results that correlated well with the L1210 system and with the spontaneous AKR leukemia. It is suggested that in order to establish a more precise correlation of activity between first generation AKR and L1210 it could be desirable to test a random sampling of new chemical structures in both systems in order to determine whether the first generation AKR system will identify structures as active that are not active in the L1210 system.

The advanced leukemia L1210 system is not as sensitive as the early L1210 system to chemotherapy and the correspondence of activity between advanced L1210 and spontaneous AKR leukemia appears to be rather good. Since with both of these systems the disease is systemic when treatment is initiated, it is suggested that advanced L1210 as well as spontaneous AKR could be utilizable for detailed studies of remission induction and maintenance. It is indicated that the spontaneous AKR system may be useful not only for the study of remission induction and maintenance, including consolidation therapy, but also for the evaluation of prophylactic treatment and the study of reinduction in the event that total "cell cure" may have been established. Further correlation of drug activity in the spontaneous AKR system with clinical activity in fundamental detailed investigations may prove highly useful to clinical chemotherapy.

A final report of the above analysis is being prepared for publication.

C. The Relationship of the White Blood Cell Count to the Diagnosis and Therapeutic Response of AKR Mice with Primary Spontaneous Lymphoma. (M. Kende, A. I. Goldberg, J. P. Glynn (deceased), N. Mantel and A. Goldin)

Studies have been conducted on parameters for the diagnosis of AKR spontaneous leukemia. It was found that spleen and lymph node enlarge-

ment provided a consistent indicator for the selection of leukemic mice in their terminal stage. On the other hand a high white blood cell count was not a reliable diagnostic aid because of the virtually identical median survival time and cumulative death pattern of untreated mice with high or low white blood cell counts. Mice with high or low white blood cell counts had similar remaining life spans. They responded equally well to cyclophosphamide therapy administered alone or in combination with cytosine arabinoside with respect to the extent of decrease in spleen weight, the survival time achieved and the percent of 50-day survivors. Also no relationship was observed between spleen, liver or thymus weights and the white blood cell count.

D. Development of Animal Tumor Systems Which are Predictive for Empirically Derived Clinical Combinations. (S. Vadlamudi, M. Padarathsingh, R. Johnson, N. Mantel and A. Goldin)

Most of the multiple drug combinations used in the treatment of human cancer were developed through clinical research and have little or no correlation with animal systems. The establishment of animal models which respond to combinations in a manner similar to human cancer (i.e. the combination is more active than single agents) would be useful. With such a system experiments could be performed to optimize the dosage schedule of the various drugs in the combination and to determine whether substitution of one of the drugs with a related agent or addition of another drug to the combination could increase the therapeutic efficacy without markedly enhancing toxicity.

Studies were performed in leukemia P1798 with the remission induction and maintenance schedule of vincristine plus prednisone followed by methotrexate used widely in acute lymphocytic leukemia. Best results were obtained when methotrexate was employed following vincristine or following vincristine plus prednisone. The data indicate that methotrexate was not too effective for induction but that the drug was useful for remission maintenance when tumor remission was initially induced by vincristine or vincristine plus prednisone.

The "MOPP" regimen is the primary approach to treatment of stage III or IV Hodgkin's Disease. This combination, consisting of nitrogen mustard, vincristine, procarbazine and prednisone has been assessed in mice bearing leukemia L1210. Only one of these agents, procarbazine, has any notable therapeutic effect on this tumor system. None of the four-, three-, or two-drug combinations proved to be more effective than procarbazine alone. Thus leukemia L1210 cannot be used for detailed evaluation of the MOPP combination.

E. Chemotherapeutic Model with the K-B-N-Cl-6 Non-Producer Clone of In Vitro MSV-Transformed BALB/3T3 Cells. (R. Johnson, M. Kende, T. Brescia, Y. Ikawa and A. Goldin)

An initial evaluation was made of the in vivo growth characteristics and chemotherapeutic response of an adenocarcinoma produced by primary implantation of a non-producer clone of in vitro MSV-transformed

BALB/3T3 cells. The estimated doubling time for this tumor following ip inoculation in syngeneic mice was approximately two days. Growth of the sc tumor followed Gompertzian kinetics with mass doubling times increasing from 0.8 days for tumors weighing 100 mg to 6.3 days when tumors reached a mass of 1 gm.

Treatment of mice with early or advanced disease with cyclophosphamide was highly effective as measured by prolongation of lifespan or inhibition of local tumor growth. Apparent cures were obtained after single dose or intermittent therapy with this agent. BCNU was effective in prolonging survival of tumor-bearing mice but did not produce long-term survivors. 5-Fluorouracil treatment resulted in a moderate increase in survival time of mice with ip tumors but was ineffective against the sc tumor. Methotrexate and bleomycin had no therapeutic activity in this tumor system.

Studies with this tumor system are continuing to determine whether it could serve as a chemotherapeutic model for human carcinoma. A wider selection of antitumor agents with diverse mechanisms and varying degrees of clinical activity are being tested to determine the predictive value of this tumor for slow-growing human neoplasms.

IV. Chemotherapy Studies

A. Drug Scheduling

1. Increased lifespan of leukemic mice resulting from alteration of the sequence of administration of BCNU and cyclophosphamide. (M. J. Straus, S. C. Choi and A. Goldin)

   Additional studies were conducted on the importance of sequencing of combinations of antitumor agents. L1210 leukemic mice were treated with a series of doses of cyclophosphamide administered at 4 or 5 day intervals and a high dose of BCNU was substituted at varying times for the regularly scheduled dose of cyclophosphamide. It was observed that earlier substitution of the high priming dose of BCNU resulted in longer survival. Early administration of a high priming dose of BCNU by lowering the tumor cell population may in addition to markedly reducing the tumor cell population also increase the percentage of cells in cycle so that subsequently scheduled doses of cyclophosphamide kill the cells more effectively.

B. Rational Approach to the Design of Effective Combination Chemotherapy. (R. Johnson and A. Goldin)

In recent years a great deal of information has been compiled on the effects of anticancer agents on the progression of cells through the cell cycle and on the cycle stage specific cytotoxicity of many of these agents. A program has been initiated in this laboratory to select theoretically synergistic two-drug combinations based on knowledge of drug effects on cell cycle kinetics. The combinations are then tested in tumor-bearing mice for therapeutic synergism and in

normal mice to determine whether toxicity is enhanced as well.

There is evidence that inhibition of protein biosynthesis by any of a number of methods halts the progression of cells through the cell cycle at the G_1/S interface. Studies of cell-cycle specific cytotoxicity have shown that cells are most susceptible to the lethal effects of alkylating agents and nitrosoureas at this same stage of the cycle. Thus concomitant administration of a protein synthesis inhibitor with an alkylating agent or nitrosourea should theoretically result in therapeutic synergism.

The combination of BCNU and emetine was used in the therapy of advanced leukemia L1210 to test this hypothesis. Emetine was chosen as it is the most active of the known protein synthesis inhibitors in animal tumor systems and shows promise in clinical cancer. This combination, utilizing a single simultaneous dose of each agent, resulted in marked therapeutic synergism as determined by both increased survival time and number of long-term survivors. In addition these experiments suggested that sub-optimal doses of emetine decreased the toxicity of BCNU. Studies of this combination in normal mice are being performed to confirm this interaction.

We will continue work in this area to determine the biochemical and cytokinetic mechanisms underlying the synergism between these two agents. Other protein synthesis inhibitors and additional nitrosoureas and alkylating agents will be studied as well.

C. Drug Resistance

1. Cross resistance characteristics of drugs. (H. E. Skipper, D. J. Hutchison, F. M. Schabel, Jr., L. H. Schmidt, A. Goldin, R. W. Brockman, J. M. Venditti and I. Wodinsky)

   The development of resistance to drugs is one of the prime limiting factors to successful chemotherapy. Also in the concomitant or sequential use of drug combinations it is important to be aware of the cross resistance characteristics of tumor cells to antitumor agents. In view of the increasing number of chemotherapeutic agents available having differing structures and mechanisms of action, it was considered desirable to compile a quick reference chart listing the published data on cross resistance, lack of cross resistance, and therapeutic potentiation. Such a chart has been developed and published and it is hoped that it will serve as a tool for preclinical investigators in the further examination of the interrelating characteristics of drugs and to the clinical investigator in the choice of drugs for concomitant or sequential chemotherapeutic schedules.

2. Attempt to prevent the origin of resistance to L-asparaginase in the treatment of mice with leukemia L5178Y. (H. Jayaram, D. Cooney, H. Milman, S. Vadlamudi, N. Greenberg, A. Goldin and J.A.R. Mead)

   Jayaram, Cooney and Milman have instituted a systematic screen

employing tumoral synthetase to select agents capable of preventing the emergence of resistance to L-asparaginase. Mice carrying leukemia L5178Y were treated concomitantly with L-asparaginase and one of three candidate compounds, namely mucochloric acid or maleimide or azotomycin. Although strong inhibition in vivo of L-asparaginase synthetase was demonstrable the emergence of resistance was not prevented.

D. Immunity and Chemotherapy

1. Chemo-immunotherapy of spontaneous AKR murine leukemia. (M. Kende, A. Airall, D. Houchens and A. Goldin)

Freeze-dried and live BCG were employed in chemo-immunotherapy of AKR leukemia, from diagnosis or following remission induction. The freeze-dried and live preparations were not effective against AKR leukemia when treatment was initiated at the time of diagnosis. Chemo-immunotherapy of AKR leukemia with BCG was effective only after remission induction. The activity was significant after remission induction with vincristine plus prednisone but it was less or completely absent after remission induction with cyclophosphamide. It is suggested that in the latter instance the failure of BCG to elicit an improved response may be attributable to immunosuppression caused by the prior treatment with cyclophosphamide.

2. Study on the effect of Bacillus Calmette-Guerin (BCG) on Moloney sarcoma virus-induced tumors. (D. P. Houchens, A. I. Goldberg, M. R. Gaston, M. Kende and A. Goldin)

The Moloney sarcoma virus was employed to investigate the actions of BCG on the tumor growth pattern and on humoral antibody production in immuno-competent mice and in mice immunosuppressed with cyclophosphamide. It was observed that pretreatment of the animals with BCG reduced the maximum tumor size and increased the number of survivors of BALB/c mice that were immunosuppressed with cytoxan and inoculated with the Moloney sarcoma virus, as compared with immunosuppressed mice which were given Moloney sarcoma virus but not pretreated with BCG. BCG administered without immunosuppression did not markedly affect the maximum tumor size of animals inoculated with MSV. BCG pretreatment was not able to overcome the immunosuppression, as measured by humoral antibody production to SRBC. The observation that pretreatment with BCG did not enhance the humoral response in mice given cytoxan suggests the possibility that cytoxan, in addition to producing a depression of general humoral response may also inhibit blocking antibody. It is possible that on pretreatment with BCG followed with treatment by cytoxan the BCG maintained or increased the cellular response but failed to overcome the suppression of the humoral (blocking) antibody. In such a case, maintenance of cellular response, accompanied by suppression of blocking antibody, could account for inhibition of tumor growth in animals treated

with BCG and cytoxan. This as well as alternative hypotheses are considered worthy of further investigation.

3. Effects of Bacillus Calmette-Guerin (BCG) on the Lewis lung carcinoma. (R. Johnson, D. Houchens, B. Zbar and A. Goldin)

The widespread clinical interest and relative lack of preclinical data on BCG prompted an evaluation of the effects of this agent on one of the standard tumor systems used by DR&D.

Enzymatic digestion of the Lewis lung tumor with pronase and DNase yielded viable single cell suspensions which produced tumors following intradermal or sc inoculation of C57Bl/6 mice. BCG or BCG cell wall vaccine (prepared according to Ribi) mixed with tumor cell suspensions resulted in an inhibition of intradermal tumor takes. Whereas Lewis lung cells admixed with cell free BCG broth produced tumors which metastasized to the lungs and killed all inoculated mice, the addition of increasing numbers of BCG cells to the tumor inocula progressively decreased the percentage of animals which developed tumors. The growth rate of the local tumor and median survival of those animals which did develop local tumors was not significantly different from controls.

Intratumor inoculation of BCG or Ribi vaccine into intradermal Lewis lung tumors when first palpable (i.e. 3-4 mm in diameter) had no therapeutic effect. In fact, the rate of local tumor growth was somewhat enhanced by this treatment.

The use of iv Ribi vaccine as an adjuvant to surgical removal of primary Lewis lung tumors had no effect on lung metastases as measured by survival time of treated animals.

These studies will continue to determine whether other schedules or routes of BCG will have therapeutic activity as primary or adjuvant treatment in the Lewis lung system. In addition, more detailed investigations of the tumor cell-BCG admixture will be performed to see if the inhibition of tumor take is related to non-specific stimulation of the immune system as proposed in other animal models or to some direct cytotoxic property of the BCG preparations.

4. Collateral sensitivity and the levels of sialic acid in sublines of L1210 leukemia. (V.V.S. Reddy, J. N. Choudhry, S. Vadlamudi, V.S. Waravdekar and A. Goldin)

It has been reported that leukemic L1210 sublines resistant to cytosine arabinoside and to 6-mercaptopurine exhibit collateral sensitivity to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). In investigation of the possible biochemical alterations responsible for collateral sensitivity a study was conducted to determine the effect of BCNU treatment on DNA synthesis and the levels of sialic acid for cytosine arabinoside and 6-mercaptopurine

resistant tumors. Resistant leukemic cells treated in vivo with BCNU failed to exhibit any selective enhanced inhibition of in vitro ^{3}H-labeled thymidine incorporation into DNA as compared with the parent sensitive line. However, an increase in levels of sialic acid was observed for cytosine arabinoside and 6-mercaptopurine resistant tumors. In vivo exposure to BCNU resulted in further increase of sialic acid. In contrast, treatment with cyclophosphamide, which did not elicit collateral sensitivity, was devoid of any effect on the levels of sialic acid. The data suggest a possible relationship between the changes in sialic acid levels and collateral sensitivity for the cytosine arabinoside and 6-mercaptopurine resistant sublines.

5. Studies on increased immunogenicity of murine lymphatic tumors. (A. Nicolin, S. Vadlamudi and A. Goldin)

The increased sensitivity of leukemia L1210 drug resistant sublines has been attributed to increased immunoresponse of the host against the resistant cells. This collateral sensitivity has also been observed following treatment with DIC of a naturally resistant subline of leukemia L1210. The question presented itself as to whether there was a necessary relationship between resistance of the tumor cells and alteration of antigenicity leading to collateral sensitivity. It has now been observed that an analog of purine precursors, pyrazole-4-carboxamide, 3-(or 5) amino (PCA), was capable of altering the immunogenicity of leukemia L1210 cells despite the fact that it exerted no antileukemia L1210 activity. The altered immunogenicity in turn resulted in collateral sensitivity to BCNU. The collateral sensitivity was also observed with 6C3HED leukemia and AKR spontaneous lymphoma suggesting that these lines can also evidence altering antigenicity.

6. Adoptive immunotherapy of mice bearing a subline of L1210 leukemia. (A. Nicolin, E. Bonmassar and A. Goldin)

It has been observed by both Mihich and Bonmassar that "collateral sensitivity" may have an immunological basis, and the associated possibility of inducing immune modification in vivo in murine leukemia and lymphoma cells by drugs has been demonstrated. Further studies were conducted which provide additional experimental evidence for altered immunological properties of a resistant line of leukemia L1210 (L1210/DIC) obtained after treatment in vivo of sensitive L1210 leukemia with 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DIC). Spleen cells collected from mice immune to L1210/DIC on adoptive transfer, protected immunodepressed mice inoculated with viable L1210/DIC. The degree of protection was a function of the ratio of the number of immune lymphocytes injected to the number of viable L1210/DIC cells. On the other hand, lymphocytes immune to L1210/DIC did not protect mice inoculated with the sensitive L1210. Furthermore, spleen lymphocytes from mice immune to the sensitive L1210, adoptively transferred into immunodepressed mice inoculated with syngeneic L1210 or

L1210/DIC, did not afford any protection. Lymphocytes from non-immune mice were also ineffective. Thus, specific cellular immunotherapy against a drug-modified subline of L1210 leukemia was demonstrated on adoptive transfer of immune lymphocytes.

7. Cytotoxic lymphocytes to a subline of L1210 leukemia. (A. Nicolin, P. Franco, E. Bonmassar and A. Goldin)

It was previously demonstrated that in vivo treatment with anti-tumor drugs may induce antigenic alterations of murine leukemias and lymphomas. Also, employing the chromium (^{51}Cr) release test, specific cytotoxic antibodies were demonstrated in sera of mice immunized with cells of a subline of leukemia L1210 resistant to cytosine arabinoside. In further studies it was observed that 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DIC) can induce antigenic alteration of leukemia L1210 (L1210/DIC) that elicits rejection of an inoculum of 10^7 viable cells in mice syngeneic to the parental L1210. It was demonstrated that spleen lymphocytes (attacker cells) from mice immunized with L1210/DIC cells were cytotoxic in vitro to ^{51}Cr labelled L1210/DIC cells (target cells). Increasing numbers of L1210/DIC cells, but not L1210 or syngeneic or allogeneic tumor or spleen cells, specifically reduced ^{51}Cr release by target cells when added to the optimal suspension of attacker: target cells. Attacker cells evidenced only a low level of cytotoxicity for the original L1210 cells, probably reflecting minor tumor transplantation antigens. The above in vitro demonstration of cellular immunity to L1210/DIC leukemic cells provides further evidence for drug induced genetic alteration of the immunological properties of a murine leukemia.

8. Antileukemic and immunosuppressive properties of abrin and ricin. (V.V.S. Reddy, J. N. Choudhry, S. Vadlamudi, V. S. Waravdekar and A. Goldin)

Two toxic plant proteins - abrin and ricin have been reported to inhibit the growth of experimental sarcomas. A study was conducted to determine the influence of these proteins on murine leukemias and on the immune response of mice to sheep red blood cells. Leukemia P388, a signal tumor for identification of active natural products, was sensitive to both abrin and ricin. Leukemias L1210, L5178Y and the Lewis lung carcinoma did not respond to treatment with these proteins. Nevertheless, the growth in vitro of L5178Y cells was inhibited at low concentrations of both proteins. Abrin and ricin exhibited moderate immunosuppressive effect in mice when injected prior to antigenic stimulation with sheep red blood cells. In contrast administration of these proteins to mice after exposure to sheep red blood cells was ineffective in suppressing the immune response.

E. A Tumor Associated Factor Depressing Antilymphoma Allograft Reactivity. (E. Bonmassar, A. Bonmassar, A. Goldin and G. Cudkowicz)

Studies were conducted on the phenomenon of "dilution escape", occurring when small numbers of lymphoma cells were inoculated into allogeneic mice. The current studies indicate that the "dilution escape" may occur where the lymphoma cells condition the allogeneic hosts so as to render them susceptible to tumor growth. The mechanism of "dilution escape" could be explained on the basis of the following model. Small numbers of antigenic tumor cells do not adequately stimulate the host's immune system but exert a slightly delayed immunodepressant activity. Subsequently when the allogeneic tumor cell population reaches a critical size so that it affords antigenic stimulation the immunosuppressed host is not capable of rejecting the tumor. On the other hand when allogeneic mice are injected with large inocula of incompatible immunodepressant tumor cells, the initial antigenic stimulation is strong enough to elicit an adequate allograft response before the immunodepressive factor can impair the immunological response of the host. It would appear that for at least two transplantable sublines of lymphoma there may have been exerted non-specific inhibition of the allograft reactivity of the host by means of a cell associated radio-resistant immunodepressive factor. The nature of this immunodepressant factor is under investigation.

F. Detection of Endotoxin in Commercial L-asparaginase Preparations. (M. Loos, S. Vadlamudi, M. Meltzer, S. Shifrin, T. Borsos and A. Goldin)

A survey was made for detection of endotoxin in commercial L-asparaginase preparations employing the techniques of complement fixation and separation by chromatography. Of fifteen L-asparaginase preparations tested 13 inhibited whole human serum complement in significant degree (12-45%). The anticomplementary activity was separable from the L-asparaginase activity on Sephadex G-200. Fractions containing L-asparaginase were therapeutic in leukemic mice and had no effect either on whole human serum complement or on human serum C1, the first component of complement. On the other hand, the fractions containing the anticomplementary activity exerted no significant therapeutic effect in leukemic mice. L-asparaginase and the anticomplementary factor were antigenically distinct and the anticomplementary factor was shown to be antigenically related to Escherichia coli lipopolysaccaride (endotoxin).

G. Comparison of Antileukemic Effect of Fluorodopan (NSC-73754), Phthorafur (NSC-148958) and 5-Fluorouracil (NSC-19893). (S. Vadlamudi and A. Goldin)

Fluorodopan and Phthorafur are antitumor agents of interest in the USSR and were obtained as part of the joint effort in cancer research. The activity of these compounds against leukemia L1210 was compared with that of 5-fluorouracil to which they are structurally related. Neither agent proved to be superior to 5-FU on three dosage schedules (Day 1 only; Q4D 1,5,9; QD 1-9). Fluorodopan had little activity in this tumor system.

These agents will be tested in 5-FU resistant tumor lines to see whether they have any unique properties.

Proposed Course of the Project

The various aspects of the project will be continued and modified in accordance with laboratory findings and clinical interest.

Further emphasis will be placed on studies of structure-activity relationships among congeners of clinically active antitumor agents. Determinations of antitumor activity and effects on cytokinetic and biochemical parameters will be made both in vivo and in vitro in these analogue investigations. Tumor sublines made resistant to parent compounds will be used to determine whether cross-resistance to analogues exists. Studies will be continued on the influence of cell synchronization on the chemotherapeutic efficacy of drugs. The information available on the effects of antitumor agents on the cell cycle will be applied to the design of more effective drug scheduling and therapeutically synergistic drug combinations.

Continued emphasis will be placed on the detailed investigation of established tumor systems such as leukemia L1210, B16 melanoma and Lewis lung carcinoma for the characterization of drugs with respect to spectrum of antitumor activity and mechanism of action. Retrospective and prospective analyses will be made of correlations of activity in these systems with clinical activity, particularly in solid tumors. Additional tumor systems will be studied with the aim of developing model systems with a high clinical predictability value for solid tumors and determination of activity for drugs of clinical interest which are inactive in leukemia L1210, P388 or Walker 256 carcinosarcoma. Emphasis will be placed on systems which allow definitive determination of antitumor activity with limited amounts of test materials.

Combined-modality approaches to the use of chemotherapeutic agents will be investigated. The effectiveness of chemotherapy in eliminating residual tumor cells following surgical removal of a primary tumor mass will be studied in metastisizing tumor systems such as the Lewis lung carcinoma. Systems will be developed for the assessment of the activity of drugs as radio-sensitizing agents. Emphasis will continue in the area of chemo-immunotherapy. Studies on the influence of drugs in altering tumor cell antigenicity will be continued. The relationship of the immune system to the phenomena of "collateral sensitivity" and "therapeutic synergism" will be investigated in more detail. Studies of non-specific and specific immuno-enhancement will be continued from the point of view of overcoming drug-induced immunodepression and improving therapy.

Detailed evaluation of drugs of potential clinical interest will be continued. New agents of unique structure and reproducible antitumor activity will be investigated with respect to biochemical mechanism of action and effect on the mammalian cell cycle. Studies will be continued to determine procedures for reducing drug toxicity and improving therapeutic effectiveness. In relation to clinical interest emphasis will continue to be placed on development of model systems for remission induction and maintenance and for the evaluation of combinations of drugs which have proven to be highly effective in various human tumors.

Various facets of programs in progress and problems of particular clinical interest will be investigated. Increased attention will be focused on preclinical investigations in liaison with clinical investigators.

Honors and Awards: (1) A.Goldin received the Ambrosiani Medal of Milan; (2) A. Goldin - Honorary membership in Hungarian Cancer Society.

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MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION
OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR DR & D

INTRODUCTION

During 1972, the Mammalian Genetics and Animal Production Section continued its functions as the source for the provision of laboratory animals. This supply program included eight species subdivided into a variety of inbred strains and outbred stocks. Ancillary activities consisted of the maintenance of disease diagnostic laboratories, investigations to ensure continuity of genetic characteristics of the biological material, and guidance and counseling to users as well as producers of laboratory animals.

With the advent of the Conquest of Cancer program, the Section's overall activities were expanded proportionately. As a result, it became necessary to accelerate the 3-stage production scheme, concurrently expand monitoring activities, and to activate additional Primary Centers.

Animal breeding programs included the primary isolator colonies, animals in isolators with defined microbial flora, barrier colonies, secondary centers in bio-containment and modified conventional environments, rodent production centers for breeder production and specific investigations, and hybrid production colonies. The workscopes of diagnostic laboratories were expanded to accommodate the increased number of specimens in order that the quantity of samples would yield statistically significant information. Standards were upgraded in line with emerging information from the field of laboratory animal care and the area of laboratory animal medicine. These specifications recognized the possibility that more precise parameters would emerge in the future for the application of biologically defined animals for research and testing programs. Laboratory investigations included the identification of normal intestinal flora in conventional animals; the determination of the continuation of genetic susceptibilities and resistances to specific microorganisms; and the development of physiologic parameters for rederived animals from a variety of inbred strains. Associated activities included conferences on the application of laboratory animals in biomedical research and improvement in laboratory animal care with particular emphasis on environmental factors; the development of biological models; "new looks" at professional and subprofessional training; and an expansion of the DR & D accreditation program. The Section continued to maintain surveillance over and provide supportive care for experimental animals of the DCT area in Building 37.

The Section's animal production program during 1972 furnished 75 to 85 categories of the eight species noted above. Variations reflected short-term use for special hybrids required for specific projects. The majority of the types produced consisted of inbred and hybrids of inbred mice. With the exception of beagle hounds, rabbits and guinea pigs, all breeding animals were provided through the Section's animal supply program. The Section also functioned as the source for hosts used for the maintenance of tumors in the tumor repositories and for the transfer of tumored animals

from the tumor banks to contractors performing drug evaluation studies.

The Section staffing consisted of one senior staff member, three professional associates, one animal contracts assistant, one clerk-typist, one animal caretaker supervisor and four animal caretakers.

The senior staff member and the three professional associates served as Project Officers and/or Assistant Project Officers for the following contracts:

BDF_1 hybrid mouse production	12
CDF_1 hybrid mouse production	6
Primary Centers for rodents	5
Primary Center for inbred rats	1
BALB/c Genetic Center	1
Inbred mouse production	1
Rodent Production Centers	7
Beagle hound production	4
Genetic Production Centers	4
Disease Diagnosis and Research	3
Histocompatibility studies	1
Testing and research laboratories	3
Standards for Care and Maintenance	1
Conferences on Laboratory Animal Medicine and the Use of Laboratory Animals	1

The senior staff member and professional staff associates served as ex-officio Assistant Project Officers for advice/guidance and counseling in animal husbandry practices for all contract-supported testing and research laboratories.

ACCOMPLISHMENTS

Animal Production

The significant increase in laboratory effort generated by the expanded cancer program and the indicated requirement to accomplish the various objectives necessitated a rapid revision of previously projected goals for the Section's activities. The total use during 1972 represented an increase of 2,419,521 animals of the eight species in comparison to the total number shipped to laboratories in 1971 (Table 1 - Appendix). The major portion of the increase was associated with requirements for CDF_1 and BDF_1 hybrids, and DBA/2, AKR (weanlings and aged), BALB/c, C3H/He and C57BL/6 inbred mice. With the exception of comparatively small groups of animals supplied to other NCI program areas, the animals were used for projects sponsored by the Division of Cancer Treatment.

Primary Genetic Centers

The number of Primary Genetic Centers was increased by the awarding of contracts, selected by competition, to additional institutions. One of those selected as a site is the Leo Goodwin Institute for Cancer Research,

Nova University, Fort Lauderdale, Florida, and the other is located in the Central Animal Facilities, Washington State University, Pullman, Washington. The workscopes of both centers include isolator maintenance of pedigreed foundation and expansion colonies, and modest sized production colonies. Both centers are demonstrating excellent progress in completing workscopes despite the fact that they are currently functioning in a first year start-up period.

The Tulane School of Medicine Center is continuing to demonstrate excellent progress. The strains of rats established in barrier-type room environments during the previous period are currently furnishing significant numbers of animals for special projects. The Principal Investigator and his professional staff have published useful data concerning physiologic parameters of several strains of inbred rats.

The Primary Center maintained by the Charles River Breeding Laboratories (CRBL), Wilmington, Massachusetts, was expanded during 1972 in order to increase the production of defined laboratory rodents. Six additional strains of mice were successfully established in the isolator environments. Task 2 of this contract was initiated in Calco, Italy, in order to produce rodents required for Contract No. NO1-CM-33720 with "Mario Negri" Institute for Pharmacological Research. The colonies demonstrated steady progress towards achieving predicted breeding population levels. Once this status is achieved, shipping costs and the hazards of making shipments from North America to Europe will be eliminated. Since one of the objectives associated with this project is to avoid epizootics, the breeding animals originate from the isolator system managed by the parent company. As a result, the numbers of weanlings available on a weekly basis are limited. However, replication in the Calco colony will serve to supplement supplies of breeding animals originating from the United States project.

The division into four tasks of the Primary Center contract with the ARS/Sprague-Dawley Company has resulted in a more flexible arrangement for program needs. This system provides a mechanism for rapid adjustment of individual segments of this contract in accordance with changes in laboratory program plans. Several additional conventional strains of rodents have been derived during 1972, and significant numbers of "clean" animals will become available in 1973.

Experiences during 1972 have served to strengthen our belief that the Primary Centers complex is the basic ingredient for the production of genetically defined healthy laboratory animals. Breeding animals issued from this system to large-scale production colonies have produced animals free of parasites and pathogenic microorganisms. A gratifying spin-off has been a marked increase in the Production Efficiency Index (PEI) and a reduction in the time required for the animals to reach screening weight.

Genetic-Production Centers

The Genetic-Production Centers produced inbred, hybrids of inbred, and outbred rodents in modified conventional environments. Facilities, air handling

systems and methodology were improved. Defined animals from Primary Centers were supplied to several Genetic-Production Centers as beginnings of a gradual conversion of all conventionally maintained colonies. As in the past, these centers continued to produce unusual hybrids for R & D studies. The numbers required per special project were insufficient for assignment to large-scale contract colonies.

The dispersion of counterpart strains or stocks among two or more centers served to minimize the effects of mechanical failures in two centers, and temporary reduction in output for several colonies.

Rodent Production Centers.

These centers, during 1972, produced weanling and aged AKR mice, and DBA/2 and C57BL/6 mice for breeding purposes, as tumor carriers, and for screening purposes. Two additional contracts for similar activities were awarded during 1972. The workscopes for these projects play dual roles in the Section's animal provision program. They readily adapt to changes in laboratory program requirements, and have proven to be ideal systems for large-scale production of rodents.

Hybrid Mouse Production

The implementation of the Conquest of Cancer Act and the rapid acceleration of the Chemotherapy program created a need for approximately double the number of BDF_1 hybrid mice used during 1971. Despite the usual phase-up time for new colonies, production goals were achieved within a period of three months. Sufficient animals were provided to all screening laboratories and, as a result, animal supply did not limit the expansion of testing capacity.

In-House Animal Colony Activities

The Section's Animal Caretaker Staff continued to provide husbandry services for DCT laboratory areas in Building 37. These activities included the conditioning of animals prior to their use for investigations and the care of animals used for DR & D and Toxicology.

Service Laboratories

The disease diagnostic laboratories were generally concerned with the routine screening of fecal, serum and/or plasma specimens. From time to time, specially selected animals, tissue, fecal samples and body fluids received microbiological, histopathological and parasitological examination. Epizootics were non-existent during 1972. The number of Salmonella spp. positives were less than 0.5%. S. typhimurium was undetected during this period although a few unusual serotypes, such as S. anatis and S. Miamia were associated with two colonies. Improvement in husbandry practices served to eliminate these organisms.

P. aeruginosa continued to be rather prevalent, but the incidence subsided during the last quarter. This decrease was attributed to the treatment of

water supplies with Sodium hypochlorite and/or Hydrochloric Acid at pH 2.5 and the application of sterile techniques. The availability of Salmonella/ Pseudomonas diagnostic laboratories at the Battelle Memorial Institute and the IIT Research Institute served several purposes. The routine screening schedules could be readily adjusted in order to make room for and focus upon an urgent incident. Reports from one laboratory could be quickly confirmed by the second laboratory.

Epizootics of Infectious Ectromelia were apparently non-existent for we did not receive requests for assistance. Interest in vaccinating mice for the prevention of this disease remains at a high level if the requests for vaccine are used as criteria. The profile of tests provided by the Mouse Pox Service Laboratory was increased with the inclusion of LDH. This service enabled us to identify colonies free from the LDH virus, particularly C3H/He mice used for Breast Cancer studies.

Reports received from the Mouse Pox Service Laboratory indicated that our isolator colonies were free of the murine viruses. We also observed that surgical/isolator derived colonies can remain free from murine viruses if they are propagated under filter covers in barrier-type environments.

The histocompatibility studies performed by Health Research, Inc., were expanded in order to accommodate animals produced by the additional Primary and Rodent Production Centers. Satisfactory results were reported for all strains with the exception of one colony of minor interest to the overall program. In this instance, only one subline appeared to be aberrant, and it will be replaced. Karyotypes were classified for L1210 susceptible and resistant lines and the B16 melanoma.

The Mason Research Institute continued to perform histocompatibility studies based upon reciprocal skin grafts for the 16 inbred rat strains produced by the various centers. Investigations to identify the Hag antigens of this species were expanded as additional rat strains were derived in the Primary Centers.

The incidence of dead and ill animals reported by consignees during 1972 was 2.916%, a decrease of 0.074% in comparison to 1971. This result is of particular significance in consideration of the fact that the number of animals supplied to contractors in 1972 exceeded those furnished in 1971 by 82%.

The Accreditation Program

The DR & D accreditation program was continued as a means to identify organizations capable of being considered as potential suppliers of laboratory animals. This information was transmitted to the Contracts Operations Branch for competitive purposes. A minimum of two inspection visits was made in order to determine whether these organizations were in compliance with the standards applied for this purpose.

Unaccredited organizations which responded to notices posted in the Commerce

Business Daily were visited in order to determine whether they could be included as potential recipients of Requests for Proposals.

During 1972, visits were made to production and research animal colonies as follows:

Primary Genetic Centers	24
Genetic Production Centers	16
Rodent Production Centers	24
Inbred Mouse Production Colonies	3
Commercial Rodent Production Colonies	27
Hybrid Mouse Production Colonies	50
Research Animal Colonies	35
Service Laboratories	14
Miscellaneous visits	50
Total	243

Task Order 6 with the National Academy of Sciences developed the following publications:

Laboratory Animal Medical Subject Headings
Users of Laboratory Animals in the United States
Selected Abstracts on Animal Models I
Selected Abstracts on Animal Models II

Additional Standards have been completed and will be published in 1973.

The Procurement Standards developed by the Ad hoc Committees and Sub-Committees furnished useful information for the biomedical community. The objectives of recently published standards were based upon anticipated upgrading of methodologies in the future.

Task Order 16 with the National Academy of Sciences sponsored the following conferences during 1972:

4th International Symposium on Germfree Research
Workshop on Distribution and Abundance of Non-human Primates
Laboratory Animal Diseases: Diagnosis and Control I
Laboratory Animal Diseases: Diagnosis and Control II

The meetings and symposia supported by this Task Order provided all members of the scientific community with the opportunities to discuss mutual problems and develop solutions or alternatives. As a result, information concerning improvements in laboratory medicine and laboratory care, biological models for specific investigations, and the value of species hitherto not used in laboratory studies were discussed and published.

Technical Assistance

The Section responded to an increase in the number of requests for information, guidance and counseling during 1972. The inquiries included assistance in

building design, animal production methodology, sources of supply for animals difficult to obtain, assistance for the alleviation of pathologic conditions, program planning, training programs, and the selection of animal care equipment for specific tasks.

The MG & APS and the Chemotherapy exhibits were presented during the annual meetings of the American Veterinary Medical Association and the American Association for Laboratory Animal Science.

PROPOSED COURSE OF PROJECT

The emphasis placed upon the animal production segments of the MG & APS program was disproportionate to the rate of progress in developing clean supportive colonies; i.e., those which furnish breeding animals for large-scale production colonies. During 1973, it is anticipated that the barrier-type colonies and the Primary Centers will become effective as sources for the conversion of conventional colonies to a healthier status. Protocols for these objectives have been developed and will be included within the workscopes of contracts.

Investigations will include evaluations of derived animals for screening purposes, improvements in diagnostic systems, the identification of suitable microflora for the adaptation of isolator animals to room environments, and efficient management of animal production colonies. Additional studies to be performed in Primary Centers will include development of systems for the derivation and maintenance of unusual strains and stocks, improved nutrition for isolator animals, and the development of physiologic parameters for isolator rodents.

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TABLE I

ANIMALS SUPPLIED DURING CALENDAR YEARS 1971 AND 1972

Inbred Mice	1972	1971	+ Increase - Decrease	
A/HeCr	14,359	9,826	+	4,533
A/JCr	2,135	1,769	+	366
AKR/Cr	249,637	201,239	+	48,398
AKR/Cr - aged	114,558	--	+	114,558
AKR/Cr - pregnants	10	6	+	4
AL/N	1,918	1,764	+	154
AL/N - pregnants	--	8	-	8
BALB/cCr	216,015	134,458	+	81,557
BALB/cCr - pregnants	7,206	11,897	-	4,691
BALB/cCr - litters	348	265	+	83
BALB/cJCr	676	256	+	420
BRVR/SrCr	288	--	+	288
BRVR/SrCr - litters	5	5		--
BRVS/SrCr	926	58	+	868
BSVR/SrCr	287	--	+	287
BSVS/SrCr	959	38	+	921
BSVR/SrCr - litters	4	12	-	8
B10.A/Cr	3,975	1,674	+	2,301
B10.A(2R)/Cr	642	310	+	332
B10.A(5R)/Cr	6,569	3,590	+	2,979
B10pd/CzCr	1,847	641	+	1,206
B10.129(5M)/Cr	10	319	-	309
CBA/JCr	3,390	3,714	-	324
CBA/JCr - pregnants	4	9	-	5
CFW/PlCr	1,496	1,215	+	281
C3H/BiCr	--	139	-	139
C3H/BiCr - pregnants	--	2	-	2
C3H/HeCr	83,233	47,813	+	35,420
C3H/HeCr - pregnants	67	38	+	29
C3H/HeCr - aged	1,753	--	+	1,753
C3Hf/HeCr	6,737	3,456	+	3,281
C3Hf/HeCr - pregnants	5	--	+	5
C57BL/6Cr	207,038	113,553	+	93,485
C57BL/6Cr - pregnants	136	164	-	28
C57BL/6Cr - litters	--	8	-	8
C57BL/KaCr	2,166	2,510	-	344
C57BL/KaCr - pregnants	--	6	-	6
C57BL/10ScCr	3,673	2,885	+	788
C57L/Cr	2,052	996	+	1,056
C57L/Cr - pregnants	34	--	+	34
C58/JCr	302	--	+	302
DBA/2Cr	113,979	98,584	+	15,395
DBA/2Cr - litters	--	14	-	14

TABLE I
(Continued)

Inbred Mice	1972	1971	+ Increase - Decrease
I/Cr	18	67	- 49
NIH/PlCr	2,906	1,413	+ 1,493
NIH/PlCr - pregnants	18	--	+ 18
NIH/PlCr - litters	1	--	+ 1
NZB/Cr	2,518	3,329	- 811
NZB/Cr - litters	1	12	- 11
NZW/Cr	1,822	1,842	- 20
NZW/Cr - litters	1	7	- 6
P/JCr	37	--	+ 37
PRI/PlCr	2,318	2,723	- 405
PRI/PlCr - litters	2	--	+ 2
SJL/JCr	5,847	6,595	- 748
SJL/JCr - pregnants	--	15	- 15
SM/JCr	2,086	1,080	+ 1,006
	1,058,172	647,856	+ 410,316
Pregnants	7,480	12,145	- 4,665
Litters	362	323	+ 39
Hybrid Mice			
$AKD2F_1$	959	1,624	- 665
BAF_1	3,534	4,937	- 1,403
BDF_1	3,221,422	1,446,831	+1,774,591
$B6AKF_1$	7,686	4,916	+ 2,770
$B6AKF_1$ - pregnants	6	--	+ 6
$B6C3F_1$	53,562	16,491	+ 37,071
$B6C3F_1$ - pregnants	6	--	+ 6
$B6C58F_1$	91	--	+ 91
$BKC3F_1$	1,751	2,654	- 903
CBF_1	8,150	6,674	+ 1,476
CBF_1 - pregnants	12	--	+ 12
$CCEF_1$	--	31	- 31
CDF_1	727,207	570,042	+ 157,165
CDF_1 - pregnants	--	3	- 3
$C58B6F_1$	254	--	+ 254
$C/StD2F_1$	210	--	+ 210
$C3C/StF_1$	132	--	+ 132
$C3fC3F_1$ - pregnants	9	--	+ 9
$C3PF_1$	339	--	+ 339
$D2AKF_1$	1,572	3,048	- 1,476
$D2C/StF_1$	83	--	+ 83
$D2PF_1$	166	--	+ 166
$PD2F_1$	170	--	+ 170
$ZWZBF_1$	1,518	1,543	- 25
$ZWZBF_1$ - pregnants	159	140	+ 19
	4,028,806	2,058,791	+1,970,015
Pregnants	192	143	+ 49

TABLE I
(Continued)

Outbred Mice	1972	1971	+ Increase - Decrease
Cr:MGAPS(S)	15,144	23,148	- 8,004
C4:MGAPS(S) - pregnants	13	--	+ 13
Cr:MGAPS(SW)	14,667	10,293	+ 4,374
Crl:(ICR)	2,400	--	+ 2,400
	32,211	33,441	- 1,230
Pregnants	13	--	+ 13
Inbred Rats			
ACI/Cr	2,022	1,985	+ 37
ACP/Cr	192	232	- 40
ALB/Cr	331	414	- 83
AUG/Cr	1,004	1,179	- 175
BN/Cr	193	316	- 123
BUF/Cr	2,063	1,341	+ 722
BUF/Cr - pregnants	4	--	+ 4
COP 2331/Cr	1,550	1,734	- 184
COP 2331/Cr - litters	1	--	+ 1
F344/Cr	93,547	75,118	+ 18,429
F344/Cr - pregnants	107	91	+ 16
F344/Cr - litters	1	8	- 7
LE/Cr	193	434	- 241
M520/Cr	1,011	766	+ 245
NBR/PlCr	5,283	3,456	+ 1,827
OM/NCr	324	717	- 393
SD/MCr	2,909	2,315	+ 594
SH/Cr	1,116	1,184	- 68
S5B/PlCr	450	545	- 95
W/FuCr	5,263	4,133	+ 1,130
W/FuCr - pregnants	10	34	- 24
W/LCr	5,266	5,348	- 82
W/LCr - pregnants	--	6	- 6
W/LCr - litters	--	2	- 2
YOS/Cr	2,541	1,514	- 1,027
ZIM/Cr	19	--	+ 19
	125,277	102,731	+ 22,546
Pregnants	121	131	- 10
Litters	2	10	- 8
Hybrid Rats			
$F344BNF_1$ - litters	1	--	+ 1
$WFBNF_1$	348	--	+ 348
Outbred Rats			
Cr:MGAPS(Gunn)	183	--	+ 183
Cr:MGAPS(OM)	38,711	34,357	+ 4,354

TABLE I
(Continued)

Outbred Rats	1972	1971	+ Increase - Decrease
Cr:MGAPS(OM) - pregnants	24	--	+ 24
Cr:RAR(SD)	18,091	18,804	- 713
Cr:RAR(SD) - pregnants	180	68	+ 112
Cr:RAR(SD) - litters	--	1	- 1
	56,985	53,161	+ 3,824
Pregnants	204	68	+ 136
Litters	--	1	- 1
Hamsters			
Cr:RGH(SYR)	56,328	38,573	+ 17,755
Cr:RGH(SYR) - pregnants	964	768	+ 196
GRAND TOTALS			
Inbred Mice	1,058,172	647,856	+ 410,316
Pregnants	7,480	12,145	- 4,665
Litters	362	323	+ 39
Hybrid Mice	4,028,806	2,058,791	+1,970,015
Pregnants	192	143	+ 49
Outbred Mice	32,211	33,441	- 1,230
Pregnants	13	--	+ 13
Inbred Rats	125,277	102,731	+ 22,546
Pregnants	121	131	- 10
Litters	2	10	- 8
Outbred Rats	56,985	53,161	+ 3,824
Pregnants	204	68	+ 136
Litters	--	1	- 1
Hybrid Rats	348	--	+ 348
Litters	1	--	+ 1
Hamsters	56,328	38,573	+ 17,755
Pregnants	964	768	+ 196
Guinea Pigs	135	123	+ 12
Rabbits	20	--	+ 20
Dogs	600	564	+ 36
Gerbils	212	83	+ 129
	5,359,094	2,935,323	+2,423,771
Pregnants	8,974	13,255	- 4,281
Litters	365	334	+ 31

ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

As the national effort on the eradication of cancer is accelerated, the expanded research, development and application will result. Three major avenues are presently being followed, radiation, surgery and chemotherapy. The two older techniques, radiation and surgery, are applied generally to the localized disease. The newer approach chemotherapy, has its strategy directed towards both the localized and the disseminated cancer. The foundation of chemotherapy is the drug itself. Consequently the search for more effective, less toxic, and broader acting agents will continue.

Objectives

The Drug Development Branch is engaged in a cooperative approach to the problems of cancer chemotherapy through the research and development of more effective chemical agents for use in humans. Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few, chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The latter category includes higher plants, marine invertebrates, insects, other animals, algae and fermentation filtrates. Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetics observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information is accumulating and greater achievement being experienced in the control of the more rapidly proliferating phase of cell growth, the search for new structural designs aimed against the slower growing tumors and of specific areas, i.e. lung, breast, kidney, colon, central nervous system, etc., will be increased.

As part of the broad Division of Cancer Treatment Program, the Drug Development Branch has three major objectives:

I. Fundamental Chemical Research

a. chemical synthesis of new compounds;

b. extraction, isolation and identification of new active agents from natural sources;

c. theoretical design for new chemical structures via structure-activity evaluations in collaboration with investigators of biochemistry, biology, toxicology, pharmacokinetics, and clinical trials; and

d. development of methodology for drug assay.

II. Worldwide Surveillance

a. information on chemicals and drugs; and

b. sources and collections of new chemicals and drugs for biological evaluation and use in the program.

III. Applied Services

a. accessioning, chemical nomenclature, development, maintenance and utilization of a computer data bank for chemical and drug information of materials entering the program;

b. the correlation of chemical structure to biological activity.

c. chemical resynthesis and production of active agents;

d. quality control and analytical evaluation of drugs and bulk chemicals for clinical and preclinical toxicological evaluations;

e. development and production of suitable dose formulations for use in clinical studies; and

f. storage and distribution of bulk chemicals and clinical dose formulations.

Implementation of Program

These objectives are carried out through the activities of the Branch's four Sections and its laboratory program which are:

I. Chemical and Drug Records Section: The objectives are to 1) assist in the worldwide surveillance and collection of new drugs and provide the program with possible sources of such agents, 2) manage the acquisition of chemicals contributed to the Program, 3) develop, maintain, and utilize a chemical data bank on materials of use to the program, 4) develop structure-activity methodology for new materials to be synthesized in collaboration with the Chemical and Drug Procurement Section and the Laboratory Research on Chemical Drug Design, 5) maintain adequate storage and inventory of bulk chemicals, and 6) coordinate the

distribution of materials to screeners and other investigators. Surveillance is also provided over industrial "Commercial Discreet" products.

II. Chemical and Drug Procurement Section: The objectives are divided into the synthesis, preparative, and quality control activities. The synthesis activities are involved with the synthesis of new compounds of diverse structures for antitumor evaluation.

The preparative activities include the process development and improvement of procedures for large scale production; the synthesis and isolation of large quantities of bulk chemicals and drugs for advanced screening, confirmational testing, pharmacological and toxicological evaluation, and clinical studies; and preparation of high purity radiolabeled materials for the chemotherapy studies. In addition, the procurement via purchase of large quantities of certain bulk chemicals and drugs needed by the Program are included in the preparative activities.

Chemical and quality control-analytical data are provided to the Food and Drug Administration (FDA) for the filing of Investigational New Drug (IND) applications.

III. Natural Products Section: The objective is the development of novel drugs from: 1) higher plants and animal (marine and insect) sources and 2) microbial sources. In support of these tasks, the Section maintains a worldwide program (partly contract and partly non-contract) for the collection of natural products, the preparation of extracts, fractionation of active extracts and the isolation and identification of the active components; and in collaboration with the Chemical and Drug Procurement Section, provides for the acquisition of large quantities of active materials needed for toxicological and clinical studies. Also, the Natural Products Section directs a program for the investigation of two groups of organisms, the actinomycetes and the fungi which have shown remarkable biosynthetic capabilities. This work requires development and chemical isolation. Surveillance of the literature is maintained as a means of obtaining new materials.

IV. Clinical Drug Distribution Section: The objective is to provide the clinical program with formulated drugs in sufficient quantity and of uniform quality. Such activity includes the development of new dosage forms for clinical investigations, the surveillance of the quality of such dose forms and the maintenance of adequate supply and distribution of such drugs to the clinical investigators. In addition, the Section provides the necessary supportive data for the filing of IND applications with the FDA.

V. Laboratory Research on Chemical Drug Design: The objective of the laboratory effort is to provide supportive basic chemical knowledge on drug design for the Division of Cancer Treatment via chemical synthesis

of compounds of interest, the identification and preparation of drug metabolites, the determination of physical-chemical properties of active materials and research in new methodology of drug formulation.

Even though the laboratory is only partially staffed, research in the synthetic, instrumental and drug formulation areas is underway. Current individual Progress Reports, Serial Nos. NCI-3580, 3581 and 3584 are presented later in this report.

Branch Staff

The Drug Development Branch is composed of a staff of 35 persons consisting of six senior professional scientists (GS-13 and above), seven other professional staff members (GS-9 and above), three PHS Commissioned Corps Officers, three Guest Workers, five Visiting Fellows, and the remainder technical and clerical staff.

The Branch is divided into four Sections and those responsible for each activity are as follows:

Drug Development Branch
Chief -- Dr. Harry B. Wood, Jr.

Chemical and Drug Procurement Section
Head -- Dr. Robert R. Engle

Natural Products Section
Head -- Dr. Jonathan L. Hartwell

Clinical Drug Distribution Section
Head -- Mr. J. Paul Davignon

Chemical and Drug Records Section
Head -- Mr. Robert B. Ing

The Chemical Quality Control Unit (Head, Mr. C. A. Hewitt) operates as an activity under the Chemical and Drug Procurement Section. The Antibiotic Unit (Head, Dr. J. D. Douros) operates as an activity under the Natural Products Section. The Chemical Laboratory (Head, Dr. John S. Driscoll) operates as an activity under the Office of the Chief of the Branch.

Operation of Program

The methods for selecting new agents for input into the primary screen is gradually changing. More emphasis is being directed toward selectivity of agents and less emphasis towards random procurement. At some undefined future time, the major input will be synthetic compounds specifically designed for anticancer effects and prepared under contract. This would result in more active analogs of existing active materials, fewer biologic tests required, and logically, the number of active agents should be higher.

Acquiring such agents may be more costly. Changes in the screening protocol should be incorporated to include differentiation of analog activity, including toxicological evaluation where appropriate.

If one were to obtain a constant number of active materials, random screening would require a much larger input of compounds than a rational approach. Unfortunately, the state-of-the-art has not reached a degree of sophistication which allows a completely rational approach to all drug design. Currently (1972), we will acquire the largest number of compounds from random sources, but an increasing number of rationally designed compounds are being generated. While the rational method is preferred, the random approach will serve a useful purpose for the foreseeable future. Random input has thus far provided a number of active compounds and certainly the greatest number of unique structures. However, as the structure-activity mosaic gradually takes shape, the choice of chemical compounds for introduction into the screen will emerge more from direct synthesis and will gradually replace random sample screening. The gradual mutation from randomization to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the literature and other related programs. Consideration is directed toward antitumor structure-activity relationships with emphasis on new and promising structural classes and de-emphasis or elimination of adequately studied classes or non-productive structural classes.

Analog synthesis will be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems.

Synthetic analogs of known active drugs may possess superior properties because of better transport, greater resistance to degradation or greater binding to appropriate enzyme sites, produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modification could also introduce new chromophore groups to aid in determining by analysis the drug level in the host. Analogs would also be used in mechanistic studies, and biochemical investigations which include areas of drug distribution, drug metabolites, enzymatic activity and the route and rate of excretion.

New chemical structures or analogs, developed through biochemical, cell cycle kinetics studies, pharmacological and pharmaceutical rationales and biological activities demonstrated in non-cancer program, should provide additional input to the directed synthesis approach. Plans are underway to group together those agents which have shown no cross-resistance and determine if there are any structural similarities for future analog design. The same study will be made on those agents which have shown potentiations in classical biologic and/or biochemical screens for new drug synthesis.

In addition to the search for "mitotically linked drugs", directed synthesis should also be aimed at affecting other phases of the cell cycle than the synthetic (S) phase or the mitosis (M) phase. The search for agents which

have a high degree of organ localization should be undertaken and compounds containing this property should be considered as potential leads for chemical modification. Such specific localizing agents have been encountered with compounds containing the nitroso-moiety which may penetrate the blood-brain-barrier. A program has been developed to synthesize CNS active antitumor agents. Also, drugs localizing in the lung, kidney, colon, prostate, etc., will be considered in modifying their chemical structure with the objective of introducing antitumor properties. The increasing awareness that many drugs may possess carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic development may provide improved methods of synthesis which would achieve economic advantages in the production of larger amounts of drugs for the clinic.

The Branch is keenly interested in new approaches to drug design. One such approach being exploited is the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives to define the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as, obtaining the physical-chemical measurements and the fact that the physical-chemical measurements are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

The Branch is continuing its search for natural products, which often provides an invaluable source of medicinal agents and new chemical structures. Plants continue to provide many extracts with significant tumor-inhibitory activity. It is important that the compounds responsible for this activity be isolated and characterized. Some will be useful in chemotherapy, others will be useful as tools in elucidating selective growth-regulating mechanisms and as templates for syntheses of more effective agents.

Microbial products have provided many highly novel antibiotics with tumor inhibitory activity. Investigations of animal and insect products have been initiated and there is every expectation to believe these will also provide new active agents.

Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations. The random but unique structures acquired from natural products--higher plants, fermentation products, algae, marine and insect products--will continue to be explored.

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of "Commercial Discreet" data must be ever present. A total of 2542 individual suppliers, of which 1144 are industrial suppliers, have contributed samples to the Program. During 1972, there have been 202 new non-industrial suppliers and 89 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers many manterials have been supplied from foreign countries. The increasing number of contributors is an index of the enthusiasm for the Program by the scientific community.

The Branch's continuous international surveillance for the acquiring new agents, synthetic and natural products, has provided over 299 foreign suppliers from industry, academic institutions and governmental and research organizations. Close contact is maintained with these and other potential new suppliers to provide interchange of chemical data and biological information on potential chemicals and drugs of interest to NCI and the scientific community-at-large. International visits were made by Dr. Harry B. Wood, Jr. and Dr. Jonathan L. Hartwell during this period to the following:

Dr. Roger Smith, University of the South Pacific, Suva, Fiji.

Dr. H. G. Gaudin, President, Auckland Division, Cancer Society of New Zealand, Auckland, New Zealand.

Dr. Bruce Cain, New Zealand, Cancer Research Laboratory of Auckland Division, Cancer Society of New Zealand, Auckland, New Zealand.

Dr. R. N. Stevenson, Secretary, Auckland Division, Cancer Society of New Zealand, Auckland, New Zealand.

Dr. A. K. Sutherland and Mr. Kenneth Dawes, Institute for Medical and Veterinary Research, Melbourne, Australia.

Dr. Claude Culvenor, Dr. John Loder and Dr. John Edgar, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia.

Dr. Charlson, Dr. Trainor and Mr. H. T. Howard, Macquarie University, North Ryde, Australia.

Dr. Howard, Sydney Technological Institute, Sydney, Australia.

Professor S. Angyal, Dr. Eade and Dr. Gallagher, University of New South Wales, Sydney, Australia.

Professor E. Ritchie and Dr. Sternhell, Sydney University, Sydney Australia.

Professor A. K. Kiang and Dr. A. Wan, University of Singapore, Singapore, Republic of Singapore.

Dr. K. K. Tan, Vice President of the Singapore Cancer Society, Singapore, Republic of Singapore.

Mr. K. T. Quek, Manager, Quek Shin & Sons, Ltd., Singapore, Republic of Singapore.

Dr. A. Hameed and Dr. Jalil, Institute of the History of Medicine and Technology, New Delhi, India.

Dr. T. A. Venkitasubramanian, Dr. H. Raj, Dr. H. P. Prasanna and Dr. S. Gupta, University of Delhi, New Delhi, India.

Dr. P. N. Waiti, Director General, Indian Council of Medical Research, New Delhi, India.

Dr. M. L. Dhar, Dr. M. M. Dhar, Dr. N. Anand and Dr. Gupta, Central Drug Research Institute, Lucknow, India.

Dr. Wlodzimierz Gustowski, Polish Academy of Science, Warsaw, Poland (Visiting Central Drug Research Institute, Lucknow, India).

Dr. D. J. Jussawala, Dr. M. B. Sahasrabudhe and Dr. M. P. Chitnis, Tata Memorial Cancer Centre, Bombay, India.

Accomplishments

The Drug Development Branch provides a steady flow of materials into the Program. These materials consist of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point II). A number of Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Table I).

In comparing 1972 with 1971, the data in Table I reflects a tripling in the number of crude plant extracts entering the Program. The number of crude extracts in the fermentation product category had doubled, while there has been a slight increase in the number of animal products. There has been a one-third increase in the number of synthetic materials entering the Program.

Since the greatest number of active materials in the past has come from the synthetic category, it is expected that as the input in the Program increases, the total number of active materials will also increase. This has been the case as shown in Table I. There were 375 confirmed activities in 1972, an

increase of 164 in comparison with 1971. Of these 375 confirmed actives, 356 were synthetic, 16 plant products and 3 fermentation products. During 1972, there was a decrease of 10 agents over 1971 passing Decision Point II (approved for toxicology and as candidates for clinical trial). Of the 9 new Decision Point II compounds, 5 were synthetic, 4 were plant products and there were no fermentation or animal products. There were five IND drugs filed for clinical use with the FDA. Of the five there were 3 synthetic, 1 was a plant product, and 1 was a fermentation product. There is every reason to believe these numbers will increase significantly during 1973.

Of the 15,770 new crystalline materials added in 1972 to the Program, 1162 compounds (7.4%) were acquired under contract, 2075 compounds (13.2%) were purchased, 4763 compounds (30.2%) were voluntarily submitted from industrial sources, 7770 compounds (49.2%) were from non-industrial suppliers. Of the total, 3460 new materials were acquired by field collection trips. During this period, there were 356 confirmed active synthetics, five selected for preclinical toxicology and three IND's filed (Table I).

Of the 10,434 plant extracts tested, approximately 82% were from contractors, and 189 crude extracts were active. One hundred and eight new crystalline materials were isolated from plant extracts and 16 have shown confirmed activity (Step III). During this period 4 materials of plant origin were selected for preclinical toxicological evaluation and one IND was filed (Table I).

From 2712 fermentation extracts, 204 demonstrated activity. Twenty-eight were isolated in crystalline form and 3 have shown confirmed activity (Step III). No fermentation products were selected for preclinical toxicological evaluation (Decision Point II), and one IND was filed (Table I).

A relatively new area of exploration in the search for new drugs has been the investigation of animal products. Of the 1669 crude animal products, 54 have shown activity. No crystalline materials have been isolated during this period, none has shown confirmed activity (Step III) and none were approved for Decision Point II or filed for an IND (Table I). There is every reason to believe that this new area will provide unique drugs for use in treating cancer.

The area of development and production of dosage forms for clinical use has continued to operate at a high level of effort. Production of parenteral products by formulation contractors increased by approximately 64% over 1971. In 1972, 17 projects for formulation development were assigned to pharmaceutical contractors. Many of these projects have been completed and the resultant experimental dosage forms have been submitted for further preclinical testing. Seven of these dosage forms were cleared for clinical distribution. Additionally, modifications requiring IND supplements were made on 11 existing formulations.

The production record achieved by formulation contractors accounts for 1,489,870 compressed tablets; 214,630 hard gelatin capsules and 504,626 vials.

A total of 935,353 drug items (vials and bottles) were received in 1972 and 852,193 items were distributed to clinical investigators throughout the world during that same period. An average inventory of 135 different compounds involving approximately 204 different formulations has been maintained.

In certain situations it is desirable to evaluate more than one drug or a drug and placebo under the same conditions. This is done by "double-blind" clinical studies which require that the materials used are to be formulated in dosage forms that cannot be differentiated from each other except by code. There are 19 coded studies on inventory.

The Branch upon request, has reviewed and commented upon 146 Grant Proposals. The opinions expressed have been used extensively by the Grant Study Sections in their evaluation of the Proposals. Such comments may include the number of materials submitted for testing by the grantee, indicating those compounds showing promise as potential antitumor agents.

Also upon request, the Branch has reviewed 4 patent applications. The applications are reviewed as to novelty, expected utility and need to protect the public's interest by patent. The opinions and recommendations are used by the NIH Patent Branch in their consideration of the application.

The Branch manages a total of 51 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately 8.5 million dollars and covering the major areas as shown under the primary responsibilities of the Branch. Of the 51 contracts, 48 are located in the continental United States and three are foreign contracts.

The Branch's laboratory operation has concentrated its synthetic efforts during the past year on aminosugar derivatives and the chemistry of potential central nervous system antitumor drugs. Seventeen analogs of NSC-50741 [β-D-ribofuranoside, methyl-5(2-chloroethylamino)-5-deoxy-2,3-O-isopropylidene-hydrochloride] were synthesized to determine the structural parameters which impart activity to the mixture produced when NSC-50741 is thermally degraded. Over 60 synthetically degraded fractions were also tested. While only marginal L1210 activity was found among the synthetics, good (T/C >150%) activity was obtained among the fractions. In separate CNS structure-activity studies, 5 hydantoin nitrogen mustards and 16 new phenothiazine derivatives, designed for a Hansch-type study, were prepared and characterized. Two additional structure-activity projects were completed (quinone-antitumor activity and rifamycin-antibacterial activity) and one large project (CNS drugs as antitumor agents) was initiated. A total of 64 compounds were prepared, characterized and submitted for biological evaluation by the synthesis group.

Analytical chemical research centered on the development of micro-NMR techniques. Using o,p-DDD (NSC-38721) as a model compound, it was found that a usable spectrum could be obtained from 15 micrograms of drug using Fourier transform NMR spectroscopy. A useful gas chromatographic collecting device was also developed. This allows GLC separation of components in a mixture with NMR identification of the microgram quantities collected. Octanol-water

partition coefficients and ring N-H proton identification in purine derivatives were also studied.

Program Review

The Branch sponsored an in-depth review of the Synthetic Chemistry Contract Program. The committee consisted of top medicinal and organic chemistry. The primary purpose of this review was to provide discussion on the following questions:

1. Should the NCI Synthetic Chemistry Program focus entirely on rational approach or should there be some exploratory synthesis looking at new structural classes?

2. (a) What types of compounds presently being explored should be deleted?

   (b) What types of compounds need expanded effort?

   (c) What new areas of chemistry not yet explored are ready to be evaluated?

3. What criteria can be used to decide whether and when there is a point at which synthetic effort in a selected area should be decreased or deleted?

4. What information is available for the rational design of compounds for synthesis?

5. How can the NCI Synthetic Chemistry Program best utilize outside chemical expertise for advice and in-put?

The following major recommendations were made by the committee:

a. The National Cancer Institute's Synthetic Chemistry Contract Program should encompass both rational approaches and exploratory syntheses of a more random nature which includes the investigation of new structural classes.

b. The need for greater interaction between chemists and biologists in the evaluation of drug activity, as well as more rapid transfer of results from biological and biochemical screens to the chemists were considered essential for drug design and structural modification.

c. The allocated monies for the Synthetic Chemistry Contract Program are insufficient to accomplish the required goals and that a substantial increase in funds should be made available to the program.

A number of additional recommendations were made, including consideration of various types of compounds presently being explored by the program. In addition, considerable thought was given to the possible means by which the Synthetic Chemistry Contract Program of the National Cancer Institute can best utilize chemical expertise and a number of suggestions have been made.

In summation, the committee recognized the importance of the chemistry portion of the Chemotherapy program, and wholeheartedly endorsed the fundamental concepts of the Synthetic Program.

The two day meeting (September 25-26, 1972) was held at the Cherry Hill Inn, Cherry Hill, New Jersey. The Review Committee consisted of the following individuals:

Dr. George Buchi
Professor of Organic Chemistry
Department of Chemistry
Massachusetts Institute of Technology

Dr. Joseph H. Burckhalter
Professor of Medicinal Chemistry
College of Pharmacy
University of Michigan

Dr. Nelson J. Leonard
Professor of Chemistry
Department of Chemistry
University of Illinois

Dr. Everette L. May
Head, Section on Medicinal Chemistry
Laboratory of Chemistry
National Institute of Arthritis and Metabolic Diseases, NIH

Dr. George R. Pettit
Professor of Organic Chemistry
Department of Chemistry
Arizona State University

Dr. Gardner W. Stacy
Professor of Organic Chemistry
Department of Chemistry
Washington State University

Dr. Martin Styles
Professor of Chemistry
Department of Chemistry
University of Michigan

Dr. D. Stanley Tarbell
Distinguished Professor
Department of Chemistry
Vanderbilt University

Dr. Alan C. Sartorelli, Chairman
Professor of Pharmacology
Yale University School of Medicine

The following staff members of the National Cancer Institute were also participants:

Dr. John S. Driscoll
Dr. Robert R. Engle
Dr. Abraham Goldin
Mr. Robert B. Ing
Dr. Vincent T. Oliverio
Dr. Saul A. Schepartz
Dr. John Venditti
Dr. Harry B. Wood, Jr.

The Drug Development Branch plans to hold similar in-depth review of its contract program to sharpen focus on the objectives of the Division of Cancer Treatment. Such reviews are highly beneficial as the program expands and more emphasis is shifted toward the control of solid tumors and use of drug combinations in the treatment of cancer.

Plans are being made to hold a one day symposium in May on methodologies and approaches in structure-activity correlations. An overview of some 1700 quinone compounds, which have been evaluated in the program, is also being scheduled for this spring.

The total contract funding for all of Drug Development Branch related tasks during FY 73 was $8,416,315. Table IV provides a breakdown of these funds as categorized by the Division of Cancer Treatment's Linear Array Code. The Branch projects showing the distribution of effort among the contractors are given in Table V. The contractor's effort among various Branch projects is given in Table VI.

Summary of Branch Achievements

In summary, the following were achieved during the calendar year of 1972 by the Drug Development Branch:

1. A total of 30585 new substances (Table I) was obtained for screening or other evaluation;
   a. 15634 synthetics
   b. 2712 antibiotic cultures
   c. 10434 plant materials
   d. 1669 animal materials
   e. 136 other crystalline materials
2. A total of 1356 compounds were prepared under contract;
   a. 194 were resynthesized by the preparation laboratory contracts
   b. 1125 were synthesized as new compounds from synthesis and prep lab contracts
   c. 37 were from the natural product laboratory contracts
3. Nine new substances were assigned to Decision Point II (Table I and II) (Candidates for preclinical toxicology);
   a. 4 plant products
   b. 5 synthetic compounds
4. Five drugs were assigned IND numbers (Table I and III);
   a. 1 fermentation product

b. 1 plant product

c. 3 synthetic compounds

5. Three hundred seventy five compounds were assigned to Step III (Table I);

6. Analytical-quality control determinations were made on 303 different lots of 104 different compounds representing 204 lots of bulk compounds, and 89 lots of clinical formulations and 10 carcinogenic standards;

7. Thirteen radiolabeled compounds (Table X) were procured;

8. Seven new dosage forms were developed and eleven existing dosage forms were modified;

9. Approximately 5,474,204 formulated units were procured;

   a. 4,204,870 compressed tablets

   b. 326,130 hard gelatin capsules

   c. 943,204 vials

10. Fifty-one contracts (some joint contracts) were supervised by the Branch;

    a. 17 synthesis laboratories

    b. 10 preparation laboratories

    c. 9 plant and animal products

    d. 5 drug formulations

    e. 3 fermentation products

    f. 2 analytical-quality control

    g. 2 radiolabel laboratories

    h. 3 miscellaneous

11. There were 146 Grant Proposals and 4 Patent Applications reviewed and commented upon;

12. Samples were received from 89 new industrial concerns;

13. Materials were received from 202 new non-industrial sources;

14. Sixty-two manuscripts by contractors were cleared for publication;

15. Nine manuscripts were published by the Branch staff;

16. Two scientific seminars were sponsored;

17. Two workshops involving the Preparation Laboratories and the Analytical - Radiolabel Laboratories were sponsored; and

18. A major review of the Synthesis Program was held.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT [1]

	CRUDE EXTRACTS NO. SUBMITTED		CRUDE EXTRACTS NO. ACTIVE		CRYSTALLINE MATERIAL SUBMITTED		STEP III [2]		DECISION POINT II [3]		IND FILED	
	1971	1972	1971	1972	1971	1972	1971	1972	1971	1972	1971	1972
Fermentation Products	1215	2712	191	204	26	28	7	3	4	-	2	1
Animal Products	1203	1669	-	54	-	-	-	-	-	-	-	-
Plant Products	3015 [4]	10434	79	189	85	108	9	16	3	4	-	1
Synthetics	-	-	-	-	10040	15634	195	356	12	5 [6]	6	3 [5]
Total	5433	14815	270	447	10151	15770	211	375	19	9	8	5

(1) Based on calendar year 1972.

(2) Showing confirmed activity.

(3) Selected as candidates for preclinical toxicological evaluation and clinical trial.

(4) Individual species.

(5) Two were synthetic modification of plant materials.

(6) One was a synthetic modification of a fermentation product.

TABLE II

COMPOUNDS PASSING DECISION NETWORK II A

NSC NO.	COMPOUND NAME	SOURCE
104801	Cytembena	Synthetic
139490	Homofolic acid, 5,6,7,8-tetrahydro-5-methyl-	Synthetic
141633	Homoharringtonine	Plant
142982	Hycanthone monomethanesulfonate	Synthetic
143057	Quinaldine, 4,4'-(hexamethylenediimino)bis[6-amino-, dihydrochloride	Synthetic
143414	Rifamycin, 4-benzyl-2,6-dimethyl-	Synthetic (1)
153858	Maytansine	Plant
163062	Triptolide	Plant
163063	Tripdiolide	Plant

(1) Synthetic modification of fermentation product.

TABLE III

IND'S FILED

NSC NO.	COMPOUND NAME	SOURCE
58514	Chromomycin A	Fermentation
68075	Thalicarpine	Plant
122819	Epipodophyllotoxin, 4'-demethyl-, 9-(4,6-O-2-thenylidene-β-D-glucopyranoside)	Synthetic *
141540	Epipodophyllotoxin, 4'-demethyl-,9-(4,6-O-ethylidene-β-D-glucopyranoside)	Synthetic *
404241	Adenine, 9-β-D-arabinofuranosyl-	Synthetic

* Synthetic modification of plant materials.

TABLE IV

DISTRIBUTION OF DDB CONTRACT PROJECTS BY DCT-ARRAY[1/]

ARRAY	LINEAR L	SUPPLEMENTARY S	EXPLORATORY E	TOTAL	PERCENT
1XX	$4,564,775	0	$38,940	$4,603,715	54.70
2B1	192,146	0	0	192,146	2.28
2B4	872,817	0	0	872,817	10.37
3X1	292,531	0	0	292,531	3.48
3X2	2,381,973	0	0	2,381,973	28.30
4X2	73,133	0	0	73,133	0.87

PROJECTS

ARRAY CODE	PROJECT	FUNDNG	PERCENT
L-1XX	Synthetics	$2,641,929	31.39
	Plant Products	978,744	11.63
	Fermentation	725,052	8.61
	Marine & Anthropods	46,896	0.56
	Storage & Distribution	172,154	2.05
E-1XX	Structure-Activity	38,940	0.46
L-2B1	Formulation-Research & Development	192,146	2.28
L-2B4	Procurement	811,334	9.64
	Storage & Distribution	61,483	0.73
L-3X1	Radiolabel Procurement	292,531	3.48
L-3X2	Production	1,269,979	15.09
	Formulation Production	660,164	7.84
	Quality Control	439,533	5.22
	Storage & Distribution	12,297	0.15
L-4X2	Radiolabel Procurement	73,133	0.87
	TOTAL:	$8,416,315	100.00%

1/ Funding based on amount negotiated for FY '73, if any, or on amount obligated in FY '72 as of March 31, 1973 DCT Monthly Report of Contract Commitments, or on new or renewal proposed budget.

TABLE V

DDB PROJECTS - DISTRIBUTION OF EFFORT AMONG CONTRACTORS

PROJECT (LA Code)	CONTRACTOR (CONTRACTOR NO.)		FUNDING
I. New Agent Acquisition			TOTAL: $4,431,561
A. Synthetics:			$2,641,929
Collections: (L-1XX)			
Mario Negri	(33720)	$ 24,838	
Starks C.P., Inc.	(72-3705)	135,509	
Synthesis: (L-1XX)			
Ash Stevens, Inc.	(71-2314)	69,867	
U. British Columbia	(72-3223)	79,780	
Central Drug Res. Inst. (India)	(PL-480)	13,621	
Collaborative Research, Inc.	(66-506)	16,916	
Collaborative Research, Inc.	(71-2326)	49,343	
Dow Chemical Company	(71-2313)	90,509	
Dow Chemical Company	(33724)	150,169	
University of Georgia	(71-2311)	36,574	
University of Michigan	(69-2004)	50,000	
Microbiological Assoc., Inc.	(33728)	326,808	
Midwest Research Institute	(33743)	331,165	
Pennwalt Corporation	(71-2309)	60,000	
Univ. of Rhode Island	(71-2312)	39,450	
Sheehan Institute for Res.	(72-3719)	52,242	
Southern Research Institute	(72-3712)	561,000	
Stanford Research Institute	(33742)	398,160	
Upjohn Company	(72-3709)	35,978	
University of Utah	(72-3710)	120,000	

B. Plant Products:			$978,344
Collections: (L-1XX)			
U. S. Dept. of Agriculture	(64-1)	$328,000	
Central Drug Res. Inst. (India)	(PL-480)	11,351	
University of Hawaii	(67-748)	55,000	
Extraction & Isolation: (L-1XX)			
University of Arizona	(67-1484)	99,680	
Central Drug Res. Inst. (India)	(PL-480)	14,756	
University of Illinois	(72-2078)	85,995	
Research Triangle Institute	(69-2019)	128,755	
University of Virginia	(71-2099)	129,000	
WARF Institute, Inc.	(67-747)	126,207	
C. Fermentation: (L-1XX)			$725,052
Bristol-Myers Company	(69-35)	341,154	
University of Illinois	(72-3208)	50,375	
Scripps Clinic	(72-2042)	23,500	
Upjohn Company	(73-3707)	310,023	
D. Marine and Anthropods:			$ 46,896
Collections: (L-1XX)			
Arizona State University	(71-2308)	15,007	
Extraction & Isolation (L-1XX)			
Arizona State University	(71-2308)	31,889	
E. Miscellaneous: (E-1XX)			$ 38,940
Pomona College	(73-3702)	38,940	

II. Procurement of Material — TOTAL: $2,446,977

A. Procurement for Pre-Clinical: (L-2B4)			$811,334
Aerojet-General Corporation	(68-1300)	$ 35,909	
Aldrich Chemical Company, Inc.	(72-3706)	134,766	
Dow Chemical Company	(71-2165)	40,008	
Merck & Company	(72-2002)	274,514	
Parke-Davis & Company	(72-3708)	103,408	
Pharm-Eco	(72-3707)	57,500	
Starks Associates, Inc.	(72-3203)	163,229	
B. Production: Toxicological & Clinical Studies (L-3X2)			$1,269,979
Aerojet-General Corporation	(68-1300)	143,638	
U. S. Dept. of Agriculture	(64-1)	72,000	
Aldrich Chemical Company, Inc.	(72-3706)	57,757	
Bristol-Myers Company	(69-35)	23,801	
Collaborative Research, Inc.	(66-506)	67,662	
Dow Chemical Company	(71-2165)	160,031	
Merck & Company	(72-2002)	276,513	
Monsanto Research Corporation	(73-3709)	198,850	
Parke-Davis & Company	(72-3708)	103,408	
Pharm-Eco	(72-3707)	57,500	
Starks Associates, Inc.	(72-3203)	108,819	
C. Radiolabel - Pharmacology: (L-3X1)			$292,531
Monsanto Research Corporation	(72-3715)	149,501	
Stanford Research Institute	(71-2162)	143,030	
D. Radiolabel -Clinical Pharmacology (L-4X2)			$ 73,133
Monsanto Research Corporation	(72-3715)	37,375	
Stanford Research Institute	(71-2162)	35,758	

III. Analytical-Quality Control: (L-3X2) — $439,533 TOTAL: $439,533

Midwest Research Institute	(33722)	163,063
Stanford Research Institute	(33723)	276,470

IV. Drug Formulation — TOTAL: $852,310

A. Research & Development: (L-2B1)			$192,146
Ben Venue Laboratories, Inc.	(66-909)	$49,966	
University of Kansas	(72-3217)	85,000	
Philips Roxane Laboratories	(71-2139)	27,698	
University of Tennessee	(70-2084)	18,250	
Warner-Lambert Research Institute	(72-3250)	11,232	
B. Production: (L-3X2)			$660,164
Ben Venue Laboratories, Inc.	(66-909)	$449,694	
Philips Roxane Laboratories	(71-2139)	110,792	
University of Tennessee	(70-2084)	54,750	
Warner-Lambert Research Inst.	(72-3250)	44,928	

V. Storage & Distribution — TOTAL: $245,934

A. New Agents: (L-1XX)			$172,154
Microbiological Associates, Inc.	(33728)	$172,154	
B. Pre-Clinical: (L-2B4)			$ 61,483
Microbiological Associates, Inc.	(33728)	$ 61,483	
C. Toxicological & Clinical: (L-3X2)			$ 12,297
Microbiological Associates, Inc.	(33728)	$ 12,297	

TABLE VI

DDB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Development Branch	Funding of Tasks Monitored by Drug Development Branch Distribution by Project (Code)	(%)	($)
Aerojet-General Corp. (68-1300)		100	179,547
Procur. for pre-clin. studies	L-2B4	20	35,909
Prod. for tox. and clin. studies	L-3X2	80	143,638
USDA (64-1)		100	400,000
Acq. new agents-plant materials	L-1XX	82	328,000
Prod. for tox. and clin.-plant materials	L-3X2	18	72,000
Aldrich Chemical Co. (72-3706)		100	192,523
Procur. for pre-clinical studies	L-2B4	70	134,766
Prod. for tox and clin. studies	L-3X2	30	57,757
Arizona, U. of (67-1484)*		89	99,680 112,000
Acq. new agents-plant ext. & isol.	L-1XX	89	99,680
Arizona State U. (71-2308)		100	46,896
Acq. new agents-marine & anthropod coll.	L-1XX	32	15,007
Acq. new agents-marine & anthropod ext.&isol.	L-1XX	68	31,889
Ash Stevens, Inc. (71-2314)		100	69,867
Acq. new agents-synthesis	L-1XX	100	69,867
Ben Venue Labs., Inc. (66-909)		100	499,660
Experimental form. devel.	L-2B1	10	49,966
Form. prod. for clin. studies	L-3X2	90	449,694
Bristol-Myers Co. (69-35)*		46	364,955 793,381
Acq. new agents-fermentation	L-1XX	43	341,154
Prod. for tox. & clin. studies	L-3X2	3	23,801
British Columbia, U. of (72-3223)		100	79,780
Acq. new agents-synthesis	L-1XX	100	79,780
CDRI (India) (PL480)*		70	39,728 56,754
Acq. new agents-synthesis	L-1XX	24	13,621

TABLE VI

Acq. new agents-plant coll.	L-1XX	20	11,351
Acq. new agents-plant ext. & isol.	L-1XX	26	14,756
Collaborative Research Inc. (66-506)		100	84,578
Acq. new agents-synthesis	L-1XX	20	16,916
Prod. for tox. & clin. studies	L-3X2	80	67,662
Collaborative Research Inc. (71-2326)		100	49,343
Acq. new agents-synthesis	L-1XX	100	49,343
Dow Chemical Co. (71-2313)		100	90,509
Acq. new agents-synthesis	L-1XX	100	90,509
Dow Chemical Co. (33724)		100	150,169
Acq. new agents-synthesis	L-1XX	100	150,169
Dow Chemical Co. (71-2165)		100	200,039
Procur. for pre-clin. studies	L-2B4	20	40,008
Prod. for tox. & clin. studies	L-3X2	80	160,031
Georgia, U. of (71-2311)		100	36,574
Acq. new agents-synthesis	L-1XX	100	36,574
Hawaii, U. of (67-748)		100	55,000
Acq. new agents-plant coll.	L-1XX	100	55,000
Illinois, U. of (72-2078)		100	85,995
Acq. new agents-plant ext. & isol.	L-1XX	100	85,995
Illinois, U. of (72-3208)		100	50,375
Acq. new agents-fermentation	L-1XX	100	50,375
Kansas, U. of (72-3217)		100	85,000
Experimental form. devel.	L-2B1	100	85,000
Mario Negri (33720)*		20	24,838
			124,192
Acq. new agents-syn.coll.	L-1XX	20	24,838
Merck & Co. (72-2002)		100	553,027
Procur. for pre-clin. studies	L-2B4	50	276,514
Prod. for tox. and clin. studies	L-3X2	50	276,513
Michigan, U. of (69-2004)		100	50,000
Acq. new agents-synthesis	L-1XX	100	50,000

TABLE VI

Microbiological Assoc. Inc. (33728)*		28.6	572,742 2,012,012
Acq. new agents-synthesis	L-1XX	16	326,808
Acq. new agents-storage & distrib.	L-1XX	9	172,154
Procur. for pre-clin.studies-storage & distrib.	L-2B4	3	61,483
Prod. for clin. studies-storage & distrib.	L-3X2	0.6	12,297
Midwest Res. Inst. (33743)		100	331,165
Acq. new agents-synthesis	L-1XX	100	331,165
Midwest Res. Inst. (33722)		100	163,063
Prod. for tox. & clin. studies-qual.cont.	L-3X2	100	163,063
Monsanto Res. Corp. (72-3715)		100	186,876
Tox.&pharm.in large animals-radiolabel	L-3X1	80	149,501
Init.clin.pharm. studies in man-radiolabel	L-4X2	20	37,375
Monsanto Res. Corp. (73-3709)		100	198,850
Prod. for tox. & clin. studies	L-3X2	100	198,850
Parke, Davis & Co. (72-3708)		100	206,816
Procur. for pre-clin studies	L-2B4	50	103,408
Prod. for tox. & clin. studies	L-3X2	50	103,408
Pennwalt Corp (71-2309)		100	60,000
Acq. new agents-synthesis	L-1XX	100	60,000
Pharm-Eco (72-3707)		100	115,000
Procur. for pre-clin. studies	L-2B4	50	57,500
Prod. for tox. & clin. studies	L-3X2	50	57,500
Philips Roxane Labs. (71-2139)		100	138,490
Experimental form. devel.	L-2B1	20	27,698
Form. prod. for clin. studies	L-3X2	80	110,792
Pomona College (73-3703)		100	38,940
Acq. new agents-structure-activity	E-1XX	100	38,940
Research Triangle Inst. (69-2019)*		92	128,755 139,951
Acq. new agents-plant ext. & isol.	L-1XX	92	128,755
Rhode Island, U. of (71-2312)		100	39,450
Acq. new agents-synthesis	L-1XX	100	39,450

TABLE VI

Scripps Clinic & Res. Fd. (72-2042)*		50	23,500 47,000
Acq. new agents-fermentation	L-1XX	50	23,500
Sheehan Inst. for Res. (72-3719)		100	52,242
Acq. new agents-synthesis	L-1XX	100	52,242
Southern Research Inst. (73-3712)		100	561,000
Acq. new agents-synthesis	L-1XX	100	561,000
Stanford Research Inst. (33723)		100	276,470
Prod. for tox. & clin. studies-qual.cont.	L-3X2	100	276,470
Stanford Research Inst. (33742)		100	398,160
Acq. new agents-synthesis	L-1XX	100	398,160
Stanford Research Inst. (71-2162)		100	189,979
Tox.&pharm. in large animals-radiolabel	L-3X1	80	143,030
Init.clin.pharm.studies in man-radiolabel	L-4X2	20	35,758
Starks Assoc. Inc. (72-3203)		100	272,048
Procur. for pre-clin studies	L-2B4	60	163,229
Prod. for tox. & clin. studies	L-3X2	40	108,819
Starks C. P., Inc. (72-3705)		100	135,509
Acq. new agents-syn. coll.	L-1XX	100	135,509
Tennessee, U. of (70-2084)		100	73,000
Experimental form. devel.	L-2B1	25	18,250
Form. prod. for clin. studies	L-3X2	75	54,750
Upjohn Co. (72-3709)		100	35,978
Acq. new agents-synthesis	L-1XX	100	35,978
Upjohn Co. (73-3707)*		38	310,023 815,849
Acq. new agents-fermentation	L-1XX	38	310,023
Utah, U. of (72-3710)		100	120,000
Acq. new agents-synthesis	L-1XX	100	120,000
Virginia, U. of (71-2099)		100	129,000
Acq. new agents-plant ext. & isol.	L-1XX	100	129,000
WARF (67-747)		100	126,207
Acq. new agents-plant ext.	L-1XX	100	126,207

TABLE VI

Warner-Lambert Res. Inst.		100	56,160
Experimental form. devel.	L-2B1	20	11,232
Form. prod. for clin. studies	L-3X2	80	44,928

Serial No. NCI 3580
1. DCT, DR&D
2. Drug Development Branch
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Chemical Research in the Development of New Anticancer Drugs

Previous Serial Number: NCI 3580

Principal Investigator: John S. Driscoll

Other Investigators: T. Hirata, A. H. Khan, S. Chu, B-L. Chu, G. Peng, H. B. Wood, Jr., F. Quinn, G. M. Hazard, N. Lomax, R. Ing, R. Engle, A. Goldin

Man-Years: Total: 6.0
Professional: 5.3

Project Description:

General Objective:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. This goal is pursued using leads developed through structure-activity correlations, the data from the random screening of compounds, and literature biomechanistic rationale.

Candidate drugs can originate either as synthetic materials or may be isolated from active natural product extracts. Natural products with proven effectiveness are also synthesized.

Specific Objectives:

1. Studies of the L1210 active decomposition products of NSC-50741.
   a. The preparation of active mixtures; the isolation and identification of components.

   b. Synthesis and decomposition of analogs of NSC-50741 to determine critical structural parameters.

2. The development of CNS active antitumor agents.

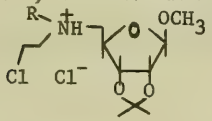
3. Structure-activity studies.

Major Findings:

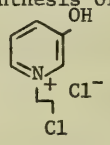
A. The Active Decomposition Products from NSC-50741

1. Preparation and fractionation of L1210 active mixtures (B-L. Chu, A. H. Khan)

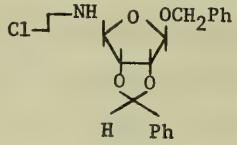
Pure NSC-50741 is inactive in the L1210 test system. It was found that upon standing for 5 years at 4°, NSC-50741 decomposed to an L1210 active mixture. The objective of this project is the acceleration of the production of the active mixture, as well as the isolation, identification, and synthesis of the active component.



NSC 50741, R = H
1a, R = CH_3
1b, R = CH_2CH_2Cl



NSC 128721



(2)

Basic investigations relating to the preparation and fractionation of decomposed NSC-50741 samples were first carried out during the period 1970-1971. This study was then suspended for 8 months until a NIH Visiting Fellow with the appropriate background was found to continue the work. Research was reinstituted in September 1972.

Although many spectroscopic and chromatographic methods have been investigated in attempts to correlate a physical parameter with L1210 activity, the only reliable measure of activity is the in vivo test. The material is not KB active.

Recent tests of the synthetically degraded material in several tumor systems gave the following data [tumor (T/C)]: L1210 (156%); B16 Melanoma (185%); P388 (225%); IC ependymoblastoma (150%); Lewis lung (123%).

It is now apparent that a number of parallel and competing chemical reactions occur when NSC-50741 is hydrolyzed to produce an active mixture. Some of the details are known from products isolated, and some can be inferred from the same data. The starting material (NSC-50741) and final pyridinium product (NSC-128721) are both L1210 inactive as is the isopropylidene deblocked material which has been isolated from the hydrolysis mixture. The logical intermediates between NSC-50741 and NSC-128721 can all be expected to be unstable towards dehydration reactions, and color changes are obvious when the sealed lyophilized product is exposed to room temperature for several days. When the white lyophilized product was exposed to room temperature for one month, either in the presence or absence of light, the resulting dark brown syrup had undiminished activity but higher toxicity. N.m.r. analysis of the brown syrup showed increased aromatic character.

Because of the delicate thermal nature of the suspected intermediates, the lyophilization conditions used were expected to be critical. Samples of the same hydrolysis mixture were lyophilized for 48, 72 and 96 hour periods at shelf temperatures of 0°, 25° and 50°. Activities decreased from T/C $>$ 150% to $<$ 125% with increasing temperature and lyophilization time.

Although active fractions (T/C 150-160%) have been isolated from the degraded mixtures by dry column chromatography, yields were low and the fractions were still mixtures. Current isolation work centers on a preliminary counter current distribution followed by partition column chromatography. Emphasis is also being placed on a reinvestigation of the effect of hydrolysis conditions on the structural and activity characteristics of the product. Using 0.1, 1.0 and 1.5N DCl/D_2O as the hydrolysis medium, the rate of production of acetone and methanol from NSC-50741 at 22° as well as the rate of formation of NSC-128721 can be followed by n.m.r. spectroscopy. The objective is the determination of optimum conditions for complete degradation of inactive NSC-50741 with no formation of the inactive final product (NSC-128721). Optimun conditions appear to be 1N DCl at 22° for 40 minutes. A lyophilized sample has been prepared under these conditions and is being screened.

2. Synthesis of Analogs of NSC-50741 (A.H.Khan)

A series of 17 analogs of NSC-50741 were prepared which were designed to answer the questions (a) is the nitrogen mustard group necessary for activity, (b) is a secondary amine necessary and (c) can derivatives of sugars other than ribose have better activity?

Compounds were screened both as pure compounds and after the hydrolysis and lyophilization procedure found to produce activity from NSC-50741. The inactivity of most of the pure and degraded samples indicate that the nitrogen mustard function is necessary for activity. A newly synthesized sample of the N-ethyl derivative of NSC-50741 (1a) had marginal L1210 activity and good (T/C 186%) P388 activity. Hydrolysis and lyophilization of this compound gave a mixture which had L1210 activity of the same magnitude but was more toxic. The two arm mustard (1b) had previously shown non-reproducible activity. A new sample was prepared to help check the tertiary vs secondary amine requirement [(b) above]. The new sample was active in the pure form (L1210 T/C 139%). A hydrolysis study on (1b) has not yet been carried out. The mechanism of action of (1a) and (1b) may be quite different from that of decomposed NSC-50741. Test results are not yet available on a number of the submitted analogs. An intense effort is currently being placed on the synthesis of the demethylated, non-glycosidic analog of NSC-50741. Catalytic hydrogenation of the benzyl analog appears to have been successful. The initial reaction in a five step sequence to synthesize an arabinose analog of NSC-50741 has been completed.

3. Synthesis of Possible Active Components from Decomposed NSC-50741 (S-Y. Chu)

A structure-activity study of the data available on aminoribofuranosyl sugars lead to the hypothesis that the key for activity might be removal of the methylglycoside blocking group. The synthesis of (2) is in progress. Both blocking groups in (2) should be removable by reduction with hydrogen over a palladium catalyst.

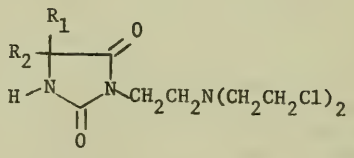
The sodium bisulfite addition product of non-glycosidic, deblocked NSC-50741 was prepared by the hydrolysis of NSC-50741 in the presence of sulfur dioxide. This compound had low L1210 activity (T/C 128%). We are currently studying the conversion of this material into compounds which are suspected to be the intermediates between NSC-50741 and NSC-128721.

Two steps in the 5 step synthesis of a xylose analog of NSC-50741 have been completed.

B. CNS Target Directed Antitumor Agents

1. Hydantoins (G. Peng)

Literature reports stating that diphenylhydantoin (DPH) crosses the blood-brain-barrier in significant amounts and concentrates in the brain tumors (but is inactive), led to the synthesis of a diphenylhydantoin nitrogen mustard (3a) which had L1210 (T/C 134%) and P388 (T/C 210%) in limited IP testing. Since CNS activity is known to be particularly dependent upon the lipophilic characteristics of a drug, we felt that the octanol-water partition coefficient of (3a) (calculated log P 4.8) might have been changed too much relative to DPH (log P 2.5) by the addition of the alkylating group. For this reason, a series of hydantoin mustards (3b-e) with a range of lipophilicities was synthesized. Three of these compounds should have log P values very similar to that determined for the parent compound, DPH. Compound 3a was resynthesized in order to obtain adequate L1210 testing. Compounds 3b-e have been prepared and submitted for testing. Results are not yet available. Work has been suspended on this project until screening results are received.



(3)

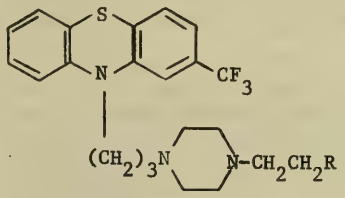
Compound	R_1	R_2	log P (calc.)
3a	Ph	Ph	4.8
3b	Ph	H	2.7
3c	$CH_2(CH_2)_3$—CH_2		2.6
DPH			2.5 (determined)
3d	Et	Et	2.5
3e	Me	Me	1.5

1. Phenothiazines (T. Hirata)

Leads obtained from DR&D rescreening and literature references suggested a program to study structure-activity relationships among phenothiazine derivatives as potential CNS antitumor agents. Two separate studies were initiated. The first involved the modification of a phenothiazine side chain based on the drug design strategy recommended by C. Hansch in Cancer Chemother. Rep. 56:433, 1972. The second approach is concerned with ring substituted phenothiazines as carriers for antitumor active groups.

a. Side-chain modification

Selected rescreening of about 25 phenothiazine derivatives showed that compound 4a and three of its derivatives were L1210 active (T/C 125-170%).



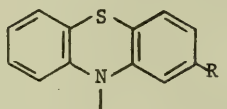
(4)

COMPOUND	R	COMPOUND	R
4a	OCH_2CH_2OH	4g	$NHCH_2CH{=}CH_2$
4b	OCH_2CH_3	4h	$N(CH_2CH{=}CH_2)_2$
4c	$OCH_2CH_2CH_2CH_3$	4i	$NHCH_2CH_2OH$
4d	OCH_2CH_2Cl	4j	$NHCH_2CH_2OCH_3$
4e	$OCH_2CH_2OCH_3$		
4f	$OCH_2CH{=}CH_2$	4k	
		4m	

Compounds 4b-m were designed with the inter-relationships among steric, electronic and lipophilic functional group parameters in mind. These compounds were prepared, characterized and submitted for testing. While insufficient data have been received to make even a preliminary judgement on activity parameters, 4b, 4e,and 4f have shown L1210 activity (T/C 127, 138, 125%). When required test data on these compounds plus another six derivatives presently under development are complete, it is planned to correlate structure and activity using the Hansch computer program which is operational within the DDB. The goal of this correlation will be the prediction of derivatives with optimum activity and therapeutic index, thus minimizing the number of new compounds to be synthesized.

b. Ring-substituted alkylating agents

Compound 5 (X=2, R=H) had been previously shown to have activity in the L1210, P388 and WA-256 test systems. The structure of almost all phenothiazine derivatives used as psycotropic agents, however, contain three rather than two methylene groups between the ring and the side chain nitrogen atoms (X=3). In addition, substitution at the 2-position (R) usually increases the effectiveness of the drug. Five derivatives of this type (Compound 5 X=3, R=H, Cl, CF_3, CH_2CH_2, OCH_3) were prepared and submitted for testing. The initial results from the first three of these compounds show them to be toxic at the only three doses studied. Additional testing is underway on these as well as the other derivatives. It is hoped that the phenothiazine ring will act as a CNS carrier for the nitrogen mustard group.



(5) $(CH_2)_xN(CH_2CH_2Cl)_2$

C. Triazoles and Purines (T. Hirata)

A previous project on the synthesis of triazoles and 5-azapurines was concluded during the past year. No L1210 activity was found among the six new materials prepared during this period as antimetabolites and methylisothiocyanate releasing compounds. A short collaborative study was carried out with the Laboratory of Theoretical Biology, NCI, which used the SAAM computer program to determine the complex kinetics of decomposition and rearrangement for an aminotriazole methylisothiocyanate adduct.

Clinical investigators had reported vein irritation problems with certain batches of formulated 6-mercaptopurine (6-MP). In order to determine whether 6-MP decomposition products were contributing to the problem, the two most likely 6-MP oxidation products (the disulfide

and sulfinic acid) were synthesized for the Laboratory of Toxicology N.C.I. Routine antitumor screening showed the disulfide and sulfinic acid sodium salt to have L1210 activity of T/C 152% and 148%, respectively.

D. Structure-Activity Studies

1. Rifamycin Derivatives (F. Quinn, J. Driscoll)

A literature report gave the antibacterial activity of 75 compounds in five bacterial systems. An attempt was made to correlate these data using the Hansch computer program at N.C.I. Good correlations were obtained in four bacterial systems. The objective of the study was to make the new improved program operational at N.I.H., and to determine whether the rifamycins would lend themselves to this type structure-activity study when the appropriate reverse transcriptase data becomes available.

2. Quinones (J. Driscoll, G. M. Hazard, H. B. Wood, Jr., A. Goldin)

A structure-activity study among the 1600 quinones which have been tested by DR&D is scheduled for submission to Cancer Chemother. Reports (Part 2) during mid-1973.

3. Psycotropic Drugs (J. Driscoll, F. Quinn, N. Lomax, R. Ing, J. Paul Davignon, B. J. Abbott)

The purpose of this study is to test two hypotheses. First-drugs with known physiological activity may be more likely to have antitumor activity than randomly selected compounds, and second - a CNS active drug (eg tranquilizer, psycoenergizer, MAO inhibitor) which is found to have antitumor activity may have a good chance of having CNS antitumor activity.

Using Usdin and Efron, "Psycotropic Drugs and Related Compounds," USPHS Publication 1589, 1967, as a drug compendia, 96 U. S. and foreign drug companies were contacted and asked to supply 400 specific CNS drugs. Sixty-four companies responded, donating over 250 of the requested drugs. Processing these compounds has just been completed. Screening will soon start in a specially designed L1210 CNS screening protocol. Since this group of compounds contains many unique structural types which are new to our program, it is hoped that this study will develop several new leads for our CNS antitumor agent synthesis program.

PUBLICATIONS

Marquez, V. E., Twanmoh, L-M., Wood, H. B., and Driscoll, J. S.: Intramolecular Cyclizations Leading to N-Bridgehead Bicyclics. J. Org. Chem. 37: 2558, 1972.

Paull, K. D., Engle, R. R., Twanmoh, L-M., Wood, H. B., and Driscoll, J. S.: The Synthesis of 9-(3,4-Dimethoxyphenoxy)-1,2,10-trimethoxyaphorine. J. Pharmaceutical Sci. 61: 1481, 1972.

Marquez, V. E., Twanmoh, L-M., Wood, H. B., and Driscoll, J. S.: Two modes of Reductive Ring Opening in 2,3-Dihydro-6,6-diphenlimidazo[2,1-b]-oxazole-5(6H)-one. J. Heterocyclic Chem. 9: 1145, 1972.

Kojima, R., Driscoll, J., Mantel, N., and Goldin, A.: Some Structure-Activity Relationships for Mitomycin Derivatives in the Treatment of Leukemia L1210. Cancer Chemotherapy Reports. Part 2, 3: 11, 1972.

Hirata, T., Wood, H. B., and Driscoll, J. S.: Rearrangement and Elimination Reactions in sym-Triazole Derivatives. J. Chemical Soc., Perkin I., in press.

Serial No. NCI 3581
1. DCT, DR&D
2. Drug Development Branch
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Analytical Chemistry of New Anticancer Drugs

Previous Serial Number: NCI 3581

Principal Investigator: J. S. Driscoll

Other Investigators: Li-Ming Twanmoh, Ilga Higbee and Harry B. Wood, Jr.

Man Years: Total - 2.2
Professional - 2.2

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;

2. establish the identity and purity of bulk and formulated drugs;

3. determine important physical and chemical properties of new anticancer drugs; and

4. study reaction mechanisms and structure-activity relationships.

Method Employed:

Emphasis is placed on modern instrumental techniques. Primary emphasis is placed on the use of high resolution NMR spectroscopy and glc as analytical tools. Chromatographic techniques, as well as infrared and ultraviolet spectroscopy, are also extensively employed.

Major Findings:

1. Studies in Combining Gas Chromatographic and NMR Methods for Structural Elucidation

a. NMR Microtechniques (Twanmoh)

NMR spectroscopy is a powerful non-destructive structural elucidation technique. Normal sample sizes required are 25 milligrams for a single scan or one tenth that amount for cumulative (CAT) scans. However, even 2 milligrams may be more than is available for some biological samples. In addition, if the effluent from an analytical gas-liquid chromatograph is to be collected for NMR analysis, then 2 mg (2000µg) is a far too large sample size for routine work. The objective of this investigation was the determination of the minimum amount of sample required to obtain a useful spectrum using proton Fourier transform NMR spectroscopy, a special technique available in the Drug Development Branch.

First, microcells were evaluated. Five different types were compared with the standard NMR tube for sensitivity, and a modified melting point tube was chosen as having the best overall characteristics. While sensitivity in the microcell for a given concentration was reduced to one-sixth that in the standard NMR tube, a 5 fold increase in sensitivity was found in favor of the microcell when samples were compared on a weight basis. The minimum volume of solvent required in various cells as well as the optimum number of transforms under many experimental conditions was determined.

A study of o,p'-DDD (NSC-38721) showed that usable spectra were obtained with 15 µg of sample in 250 µl of CCl_4. All peaks were observable including the non-aromatic AB quartet, Each of the quartet peaks represents 0.5 proton per molecule. Smaller sample sizes might be achieved if a molecule of similar molecular weight had absorptions due to more than one proton per peak. The technique is currently being generalized for different types of antitumor drugs with combination GC/NMR as a goal. The minimum quantities necessary to obtain usable NMR spectra for the following antitumor drugs were determined - cytosine arabinoside (26 µg); cytoxan (33 µg) diethystilbesterol (20 µg), and thio-TEPA (22 µg).

NMR microtechniques should complement mass spectral approaches very well for the determination of trace impurities in drugs or for metabolite studies.

b. Gas Chromatographic (GC) Studies as Applied to GC/NMR Analysis (Higbee)

There are two major GC problems to be solved in the combination approach. First, the proper column and operating conditions must be selected to affect a separation and to quantitate the process. This will be different for each mixture and is a normal GC problem. Second, a collection device must be developed that efficiently traps a few micrograms of separated sample at the GC exit port, and that also allows for efficient transfer of the collected sample to a NMR microcell. Much of our effort has been devoted to the solution of this second problem.

GC conditions were established for o,p'-DDD which was used as the model compound for this study. The separation of mixtures of o,p'-DDD and some of its common inpurities (p,p'-DDD; m,p'-DDD; p,p'-DDE and o,p'-DDE) was accomplished.

Fourteen different types of collection devices were fabricated from readily available materials and were evaluated. A disposable glass micropipet packed with a specific amount of silanized glass wool and attached to a specially modified GC exit port was optimum, giving collection efficiencies of 80-90% with 10-60 µg quantities of o,p'-DDD on column. After considerable study, 9-methylanthracene was chosen as an internal standard, and a flame ionization detector response factors for o,p'-DDD were determined so that quantitative work could be done.

2. NMR Spectral Characteristics of N-H Protons in Purines (Twanmoh)

Because of a need to characterize a number of new azapurines prepared in our laboratories, purine heterocyclic N-H NMR information was necessary. When it was found that no systematic study had been done on these important agents, the NMR characteristics of 12 xanthine and 5 azapurine derivatives were determined and correlated with structures.

3. Collaborative Studies (Twanmoh, Higbee)

In addition to investigations completed for the intramural synthesis group, short-term collaborative studies were undertaken for Microbiological Associates, Midwest Research Institute, University of Virginia, University of Arizona, Stanford Research Institute, Drug Evaluation Branch and the Laboratory of Toxicology, NCI.

4. Miscellaneous Analytical Techniques (Higbee)

Octanol-water partition coefficient and pKa techniques are now operational within the DDB laboratories.

Publication:

Twanmoh, L-M., Wood, H. B., and Driscoll, J. S.: NMR Spectral Characteristics of N-H Protons in Purine Derivatives. J. Heterocyclic Chem., in press.

CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to synthesize new compounds for antitumor evaluation, to procure large quantities for advanced screening, confirmational testing, toxicologic and pharmacologic evaluation, and clinical trial in humans, and to procure radiolabeled materials needed for pharmacologic and clinical studies.

The work of the Section is divided into the following three principal areas:

1. The Synthesis Activities, which provide new compounds for primary screening;

2. The Preparative Activities, which synthesize and isolates

   a. bulk chemicals and drugs

   b. radiolabeled materials

   for toxicologic, pharmacologic and clinical investigations, for confirmational testing and for completion of primary evaluation; and

3. Analytical-Quality Control Activities, which provide the analytical check on the identity, assay and purity of compounds and formulated drugs entering the pharmacologic and clinical programs. Solubility and stability data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section is presently composed of four professional people and one secretary. One professional person is involved full time with the quality control activities of the Section. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;

2. Leads generating from the screening program;

3. Biological and biochemical rationale;

4. Leads from the literature;

5. Compounds to explore structure-activity relationships;

6. New structural types; and

7. Agents to resolve questions on chemical and biochemical mechanism of action.

Even though major advances have been made in recent years, the treatment of cancer is still limited by the availability of useful agents. The usefulness of the majority of the present agents are limited by severe toxic side effects and/or are associated with the development of drug resistant tumors. In addition, many tumors do not respond to any of the current drugs. Therefore, a greater variety of agents with diverse structures and mechanism of action, broader tumor spectrum, and greater safety would be extremely valuable in cancer treatment. The problem of finding these improved drugs is the main objective of the contract synthesis program and its efforts are channeled into many areas of investigation. The following are some examples:

(1) A significant amount of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources, and if the structure is known, attempts are made to prepare the material by synthetic means where feasible. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be more active and interesting than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity or metabolism while retaining activity. Recognizing that natural materials are one source of interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

The clinically effective antibiotics daunorubicin and adriamycin are obtained in rather poor yields from the natural source. In addition, these compounds have dose limiting toxicologic (cardiac) properties and, therefore, synthetic work on the preparation of analogs and derivatives has continued at Stanford Research Institute. This work has been directed in the following main areas: (1) The preparation of a series of amino and carbonyl derivatives of the parent antibiotic since recent work has indicated that such derivatives may display reduced toxicity while retaining antitumor properties; (2) The preparation of more accessible synthetic analogs, mainly substituted naphthacenequinones and anthraquinones, with basic side chains that stimulate the aminosugar portion of the parent molecule; (3) The development of methodology in forming the glycosidic linkage and removal of the blocking groups on the sugar moiety; and (4) The total synthesis of adriamycin. Work in this area is continuing.

Methods for the synthesis of the clinically effective antibiotic streptozotocin have recently been developed and the large quantities of this agent needed by the program are now prepared via the synthetic route. These methods also make the modification of this interesting structure feasible. The major emphasis in this area has been on preparing analogs with modifications in the sugar portion of the molecule. Relatively few analogs of this interesting compound have been prepared and most of these have involved modifications in other than the sugar portion of the molecule. It has been speculated that the tissue specificity associated with streptozotocin is due largely to the nature of the sugar portion and by changing the sugar moiety one would expect to find significant differences in tissue specificity and/or antitumor activity. This work has been done at Stanford Research Institute and the Upjohn Company.

A limited amount of synthetic effort is devoted to the preparation of some structural moieties of the antitumor antibiotic, Bleomycin. Bleomycin is a mixture of closely related antibiotics, the chemical structures of which have recently been elucidated. The bleomycins contain polypeptides composed of a substituted bithiazole among other moieties. A few bithiazoles and related thiazoles have been prepared for evaluation at Midwest Research Institute.

The antibiotic streptonigrin has demonstrated a high degree of antiviral activity in addition to antitumor activity and is obtained in rather poor yields from the natural source. Some synthetic effort is being devoted to developing a total synthesis of this material. In addition, some simplified synthetic analogs of streptonigrin have been and are being prepared at Midwest Research Institute which contain the major features of the parent molecule.

Some synthetic effort has been and is being devoted to the development of a general and versatile synthesis of the highly complex but clinically effective dimeric Vinca alkaloids, vincristine and vinblastine. In addition to the synthesis of these clinically effective alkaloids, the synthesis of related analogs and derivatives is also being undertaken. These analogs and derivatives should provide information concerning the mechanism of action of the vinca alkaloids, their metabolism and structure-activity relationships. Also, a synthesis of these materials would provide the means of obtaining radiolabeled vincristine, vinblastine and related dimeric alkaloids for metabolism and drug distribution studies. The Vinca alkaloids are presently obtained only from the plant Vinca rosea L via a very complex and costly extraction procedure. In addition, the compounds are present in only minute quantities in the plant. The development of a synthesis for these materials has the potential of not only providing the means of obtaining analogs, but of making the materials available in larger quantities and at a lower cost. This work is being done at the University of British Columbia and a few analogs have been prepared and submitted for evaluation.

The recently completed total synthesis of the antitumor active alkaloids coralyne and thalicarpine has made it possible to undertake the preparation of analogs of these two compounds which is being done at Midwest

Research Institute. The analogs being prepared include the methylenedioxy derivative of coralyne and the nitrogen isotere (nitrogen substituted for the oxygen in the ether linkage) of thalicarpine. Other analogs of these two alkaloids are under investigation.

Several structural modifications of the L-1210 active alkaloid ellipticine have been prepared previously and some have demonstrated antitumor activity. Additional derivatives have been prepared including a variety of N-6 substituents on three pyrido[4,3-b]carbazole nuclei. The N-substituents were chosen to provide a moderate degree of reactivity towards nucleophilic groups and to give a wide range of hydrophobic and hydrophilic character. Work in this area was done at Stanford Research Institute and Central Drug Research Institute (India) and is continuing.

In addition, some effort is being extended to the preparation of some analogs and derivatives of the interesting pyrrolizidine alkaloid indicine-N-oxide. This is the only one of about 40 natural occurring such alkaloids which has shown activity in the L-1210 and P-388 leukemia test systems. The synthetic analogs are being prepared in an attempt to obtain other active derivatives and to determine the structural parameters for activity. This work is being done at Microbiological Associates, Inc.

(2) A significant amount of the synthetic effort of the Section has been directed towards the synthesis of nucleosides. The area of nucleosides has already provided a number of very interesting compounds capable of suppressing tumor growth in experimental animals and producing favorable clinical response in certain types of human cancer. Cytosine arabinoside and 5-FUDR are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to undesirable enzymatic degradation in the cancer patient.

In addition, the study of nucleoside metabolism may be expected to contribute to the design of chemotherapeutic agents with antiviral activity against tumor-inducing viruses and may help elucidate many cellular processes in the synthesis of DNA and therefore in cell division. Nucleoside antimetabolites may function by inhibiting the incorporation of normal constituents in RNA or DNA and preventing the formation of enzymes necessary for biosynthesis of the tumor nucleic acids. Also, the conversion of nucleosides to nucleotides may be structurally less restrictive than the conversion of the free base to the corresponding nucleotides.

A wide variety of structural modifications and derivatives of nucleosides has been prepared by Southern Research Institute, Stanford Research Institute and the University of Utah and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin, formycin B, and puromycin, (b) pyrrolo[2,3-d]pyrimidine nucleosides (c) tricyclic nucleosides (d) nucleosides of pyrazolo[3,4-d]pyrimidine, pyrazolo[4,3-d]pyrimidine, imidazo[4,5-c]pyridine and imidazo[4,5-b]pyrazine (e) imidazole and benzimidazole nucleosides (f) adenosylmethionine analogs (g) v-triazolo[4,5-c]pyridine nucleosides (h) "carbocyclic sugar" nucleoside derivatives (i) "carbon-carbon nucleosides" (j) "isonucleosides" (base attached

at C-2 of the sugar moiety), (k) fluorinated aminonucleosides, (l) deaza-purine nucleosides and (m) nucleosides with natural bases and fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars.

(3) Some of the synthetic effort has been devoted to the preparation of analogs and derivatives of folic acid. Methotrexate is an important clinically effective antifolate. However, resistance develops to this agent sometimes rather rapidly which limits its effectiveness. Synthetic effort in this area has been directed towards developing an antifolate which is active against methotrexate resistant tumors and/or towards obtaining compounds which retain the activity and for which the resistance problem is eliminated. The structure of the folic acid molecule has been divided into five parts, (a) Ring A (pyrimidine) of the pteridine ring, (b) Ring B of the pteridine ring, (c) the Bridge, (d) the Benzene portion (Ring C), and (e) the Amino Acid moiety and modifications have been undertaken in each area. Some of the compounds being prepared are Rings A and B deaza derivatives, the 7-aza compound, and modification in the central (benzene) portion of the molecule by replacement of the benzene ring with (1) heterocycles, (2) aliphatic chains, (3) alkylene chains, (4) cycloalkylenes, (5) other aromatic rings and (6) substituted benzene. Some compounds of this class were prepared and submitted for evaluation by Southern and Stanford Research Institutes.

Tetrahydrohomofolic acid was active against the methotrexate resistant strain of L-1210 and was selected as a potential clinical candidate, however, its poor stability proved to be a serious drawback. The 5-methyl derivative of tetrahydrohomofolic acid was prepared and shown to have better stability properties and still retains the antitumor activity. 5-Methyltetrahydrohomofolic acid is presently being obtained in large quantities for further evaluation. Additional analogs of homofolic acid are being prepared.

(4) In reviewing the compounds which have shown activity in the program the largest majority of the compounds have contained nitrogen and many have been nitrogen heterocycles. Some of the synthetic effort is being devoted to the synthesis of new nitrogen heterocycles as potential antitumor agents. The emphasis in this area has been on the preparation of bridge-head nitrogen heterocycles, especially hydropyrimidine derivatives and pyridazine compounds; imidazo[1,5-a]pyrazines, isoxazolo-[4,5-b]pyrazines and their analogs; and pyrazolotriazines, pyrazolopyrimidines, pyrazolo-pyrazines, pyrazoloimidazopyrazines, imidazopyrazines, pyrrolopurines and indenopyrimidines. Several compounds of this type have been prepared and submitted for evaluation by Dow Chemical Co., the University of Rhode Island and the University of Michigan.

(5) The class of compounds known as N-nitrosoureas are active against leukemia and some possess the ability to cross the blood-brain barrier. Three compounds of this class (BCNU, CCNU and Methyl CCNU), are currently undergoing clinical evaluation. Of special interest are the results from intracerebrally-inoculated L-1210 leukemia, Lewis Lung carcinoma, and the use of maximal single dose (one day only) treatment schedules. The testing

in rodents with some compounds of this type has resulted in a significant number of cures based on thirty-day survivals. Additional examples of this structural type have been synthesized by Southern Research Institute and submitted for antitumor evaluation.

(6) A limited amount of effort has been devoted to the isolation and synthesis of metabolites of cyclophosphamide. This clinically effective agent is itself inactive and becomes effective only after appropriate activation in vivo. Work in this area is continuing at Collaborative Research, Inc. and Southern Research Institute in an effort to determine the mechanism of activation of this active agent and to isolate and synthesize the active metabolite(s).

(7) Some of the synthetic effort of the Section has been directed towards the synthesis of compounds which potentially may localize in specific organs or parts of the body. The main effort in this area during this report period has been to develop CNS active antitumor agents. Some derivatives of ergocornine, a CNS active natural product in the lysergic acid family, have been prepared. In addition, some heterocyclic azepines and some acetylenic oxazolones and their ethynyl carbamate precursors have been prepared. The work in this area was done at the Sheehan Institute for Research and at the University of Georgia.

(8) A limited amount of the synthetic effort has been directed towards the synthesis of potential antimetabolites. One such structural type is the imidazole. Two imidazole-containing compounds have demonstrated activity against L-1210 leukemia and are undergoing clinical evaluation. In addition, some compounds of this type have been shown to have broad spectrum antifungal and antibacterial activity. Thus, additional imidazole analogs and derivatives have been prepared and are being synthesized by Southern Research Institute for evaluation as antitumor agents.

(9) In addition to the effort devoted to the synthesis of nitrogen heterocycles as new structural moieties as potential antitumor agents, a limited amount of synthetic effort was devoted to the preparation of new sulfur/oxygen heterocycles. The emphasis in this endeavor has been to prepare some 1,2,4-trithiolanes, cyclic disulfides, dithietanes and related compounds. Several compounds of these types were prepared by Ash Stevens, Inc.

(10) In order to develop the discovery of the high degree of antitumor activity in the coordination compound cis-diamminedichloroplatinum which is currently being studied clinically, the preparation of some coordination compounds of some of the transition metals has been initiated. Coordination compounds of platinum, rhodium, niobium, tantalum, cobalt, iron and nickel have been prepared and some exploratory work is being done in attempting to prepare stable, water soluble complexes of the elements gallium, indium and thallium. The work in this area has been carried out at the Pennwalt Corporation.

(11) The design and synthesis of oligo and polynucleotides as potential inhibitors of the RNA dependent DNA polymerases and other polymerases are under investigation. A series of modified and unmodified deoxy-oligonucleotide primers were prepared. These primers when complexed with the appropriate polyribonucleotide templates to form primer-template duplexes may then act as inhibitors of the polymerases. This work was done at Collaborative Research, Inc.

(12) Some of the effort of the Section has been directed towards the synthesis of DNA and receptor site oriented agents. A group of compounds designed to intercalate into DNA in the manner proposed for several antitumor drugs such as actinomycin, daunorubicin, quinacrine and probably ellipticine are being prepared. As a first approach to this concept, eight heterocyclic aromatic nuclei capable of bearing delocalized positive charges have been selected for synthesis and various basic side chains incorporated into each ring system. The work in this area has been done at Stanford Research Institute.

A common structural feature has been observed among some non-alkylating anti-leukemic agents. This structural feature consists of a triangulation composed of one nitrogen and two oxygen atoms with rather definite interatomic distances. The preparation of some condensed isoquinoline compounds designed specifically to test this observation has been undertaken and work in this area is continuing at Midwest Research Institute.

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Cancer Treatment Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position for all types of synthesis. These contractors have provided a great number of compounds with a wide variation in chemical structure.

During the past year, 9 new compounds passed Decision Point II, of which 5 were synthetic materials. These new synthetic compounds are listed in Table II.

The Chemical and Drug Procurement Section directs 17 contracts for the chemical synthesis of new compounds for the Program (Table VIII).

TABLE VIII

CONTRACT LABORATORIES FOR SYNTHESIS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ash-Stevens, Inc.	Ash	NIH-NCI-C-71-2314
British Columbia, University of	Kutney	NIH-NCI-C-72-3223
Central Drug Research Institute	Dhar	01-004-1 (PL 480)

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Collaborative Research, Inc.	Friedman	NIH-NCI-C-71-2326
Dow Chemical Company	Collins	NIH-NCI-C-71-2313
Dow Chemical Company	Trepanier	NO1-CM-33724
Georgia, University of	Blanton/Martinelli	NIH-NCI-C-71-2311
Michigan, University of	Burckhalter	NIH-NCI-C-69-2004
Microbiological Associates, Inc.	Beisler	NO1-CM-33728
Midwest Research Institute	Cheng	PH43-NCI-C-65-94
Pennwalt Corporation	Block	NIH-NCI-C-71-2309
Rhode Island, University of	Goodman/Abushanab	NIH-NCI-C-71-2312
Sheehan Institute for Research	Razdan	NIH-NCI-C-72-3719
Southern Research Institute	Montgomery	NIH-NCI-C-73-3712
Stanford Research Institute	Henry	NIH-NCI-C-71-2070
Upjohn Company	Martin	NIH-NCI-C-72-3709
Utah, University of	Townsend	NIH-NCI-C-72-3710

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not available from the original supplier or on the open market. These laboratories differ from the synthesis contract laboratories in that the synthesis contracts are devoted entirely to the preparation of new compounds with diverse chemical structures for primary screening. The preparative laboratories also perform the large scale extraction, isolation, and purification of materials from natural sources. The laboratories were selected on the basis of ability of the principal investigator, the facilities available, the capability of the company in the field of chemistry related to the Program's needs and the adaptability to synthesize a wide variety of chemical structures.

The preparation laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. Usually methods and/or

references for the synthesis of the compounds requested are supplied to the contractors by the Section. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies have also been performed by the preparation laboratories upon request. In addition, some of these contractors have provided material in formulated dose form.

The increased emphasis being extended toward the conquest of cancer has caused a major increase of the preparation activities. An increase in the number of compounds demonstrating confirmed activity will result in an increase in the number of compounds to be resynthesized for completion of screening or confirmational testing. Also, the increased effort being devoted to toxicologic and clinical studies has resulted in the need for more compounds at this level and in larger quantities. This increased effort has also resulted in the need for additional radiolabeled materials. It is anticipated that this increase in preparation activities will continue during the coming year.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs which may be needed for the Program.

(1) Bulk chemical and drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons:

(a) For clinical investigations;

(b) For preclinical toxicologic and pharmacologic evaluation;

(c) For maintaining a stock of chemicals of clinical and preclinical interest for study in the Program;

(d) For completion of screening or confirmational testing; and

(e) For use as intermediates in further synthesis.

The compounds so chosen are placed on priority lists based upon their relative importance, with the toxicologic and clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to as much as 200 kilograms. Factors governing the amounts depend upon such considerations as: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he uses.

The Chemical and Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 194 compounds totaling 542 kilograms during the calendar year 1972. Included in this group were 27 compounds scheduled for or undergoing clinical evaluation totaling 538 kilograms. In addition to the compounds resynthesized, 78 new compounds were also prepared by the preparative laboratories. During this report period the Section also purchased 18 compounds scheduled for or undergoing clinical evaluation totaling 185 kilograms.

During this report period the Section directed ten contract laboratories for the preparation of materials needed by the program (Table IX).

TABLE IX

CONTRACT LABORATORIES FOR PREPARATION

OF CLINICAL AND EXPERIMENTAL COMPOUNDS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Aerojet Chemical Co.	Jagow	PH43-NCI-C-68-1300
Aldrich Chemical Co.	Li	NIH-NCI-C-72-3706
Collaborative Research, Inc.	Friedman	PH43-NCI-C-66-506
Dow Chemical Co.	Love	NIH-NCI-C-71-2165
Merck and Co.	Babson	NIH-NCI-C-72-2002
Monsanto Research Corporation	Ellard	NIH-NCI-C-73-3709
Parke, Davis and Co.	Dice/Elslager	NIH-NCI-C-72-3708
Pharm-Eco	Draper	NIH-NCI-C-72-3707
Starks Associates, Inc.	Starks	NIH-NCI-C-72-3203
Starks Associates, Inc.	Starks	PH43-NCI-C-68-1275

(2) Radiolabeled Materials

The continued importance being placed on pharmacologic and drug distribution studies has maintained the need for radiolabeled chemicals and drugs. These agents are valuable tools in such studies and the Section coordinates their procurement and distribution.

The required labeled materials are purchased from commercial sources, if available. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. During this report period, the Section procured from all sources a total of 13 radiolabeled materials (Table X) and directed their distribution. One hundred two shipments of radioactive substances were made during calendar year 1972.

TABLE X

RADIOLABELED MATERIALS PROCURED

NSC NO.	RADIOLABELED MATERIAL	LABEL
740	Methotrexate	H^3 (3',5')
3088	Chlorambucil	C^{14} (ethylene)
68075	Thalicarpine	H^3 (6,7)
88536E	Testosterone, 7β,17-dimethyl-	H^3 (6,7)
102627	1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride	C^{14} (methyl)
118994	Diglycolaldehyde, α-(hydroxymethyl-α'-(6-hydroxy-9H-purin-9-yl)-	C^{14} (8)
126771	1,4-Naphthoquinone, 2-(3,3-dichloroallyl)-3-hydroxy-	C^{14} (1 or 4)
129943	2,6-Piperazinedione, 4,4'-propylenedi-,	C^{14} (ethylene)
141549	Methanesulfon-m-anisidine, 4'-(9-acridinylamino)-, monohydrochloride	C^{14} (9)
143414	Rifampicin, benzydimethyl-	C^{14} (38-formyl)
143647	DL-Alanosine	C^{14} (1)
145668	Cyclocytidine monohydrochloride	C^{14} (2)
145669	4(1H)-Quinazolinone, 2,3-dihydro-2-(1-naphthyl)-	C^{14} (4)

During this report period the Section directed two contracts devoted to the procurement of radiolabeled materials for use in preclinical pharmacologic and clinical studies.

TABLE XI

CONTRACT LABORATORIES FOR

PREPARATION OF RADIOLABELED MATERIALS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Monsanto Research Corporation	Yanko	NIH-NCI-C-72-3715
Stanford Research Institute	Tanabe/Leaffer	NIH-NCI-C-71-2162

Quality Control

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay and purity. All lots of bulk materials to be used in preclinical toxicological and pharmacological or in clinical studies are sent for analysis before the materials are released for use or formulation. Also, quality control data is obtained for all formulated materials prior to their release to the clinical investigators.

It should be pointed out that the Cancer Treatment program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on a routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. Also, the program on analytical studies of anticancer compounds has become involved in working out analytical methods for many of the compounds in biological media such as blood and urine.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic determinations (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric determinations (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the present of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultraviolet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound. The determination of the purity of a more complex compound is often an analytical research

problem requiring the use of a combination of sophisticated techniques, and requires taking into consideration the stability of the compound and its possible contaminants or decomposition products.

In addition to the identification and analysis of compounds under study, the analytical unit is deeply concerned with those physical and chemical characteristics of the compounds which must be considered in the handling, storage, formulation and administration of the material. Of increasing importance to the Program is solubility and stability data, which serves as a guide for drug formulation, storage, and shipping. In certain situations the decomposition of a compound, its solution or formulation may be followed by the observation of the ultraviolet absorption when the decomposition of the active moiety results in a change in absorption. For the stability of compounds which cannot be followed by this procedure, other methods must be devised. Among the other procedures used are chromatography, determination of halide ion concentration, nuclear magnetic resonance, and mass spectrometry. To validate the accuracy of these procedures, determinations are made on a sample to which known standards have been added. The analytical data on compounds of current interest is periodically reviewed and the data is assembled in the form of a Chemical Information Sheet. These Chemical Information Sheets are made available to the investigators using the material.

The need for the evaluation of the "shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications."

The Unit collects and files such data as:

1. The detailed method of preparation;

2. The supplier's analytical data sheet; and

3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the Drug Development Branch directed quality control contracts.

The assembled data is of use also in writing purchase specifications for additional quantities of the compound. In general, attempts are made to keep specifications brief by listing those requirements which are judged to best identify and assay the compounds.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open market. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

During the calendar year 1972, 104 compounds, representing 204 lots of bulk chemicals, 89 lots of clinical formulations, and 10 lots of carcinogenic reference standards have been evaluated. Each of these evaluations is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Branch and the contractors. The analytical data occasionally indicated unacceptable materials resulting in their being returned to the manufacturer for processing.

In addition, several special studies were performed. One analytical facility, Midwest Research Institute, determines the partition coefficient of compounds for use in structure-activity correlation studies. The procedure consists of equilibrating the compound in a mixture of n-octanol and water, and determining the concentration of the compound in each solvent. This often involves intensive analytical development in cases where the compound is relatively insoluble or highly soluble in one of the solvents.

Assistance was also extended to the formulation facilities with their analytical and formulation problems concerning compounds of interest to the Program.

The Section directed two analytical-quality control contract laboratories. In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE XII

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Midwest Research Institute	Haggerty	NO1-CM-33722
Stanford Research Institute	Lim	NO1-CM-33723

Miscellaneous

One contract, Pomona College (NIH-NCI-C-73-3703), is for structure-activity studies. This contract is directed towards (1) the use of the regression analysis techniques developed by Dr. C. Hansch of Pomona College to develop structure-activity correlations of antitumor agents and (2) the investigation of the factors which lead to the parabolic structure-activity relationship in enzyme-substrate systems which will be used to improve the quality of structure-activity predictions for drug-enzyme systems.

Structure-activity correlation was made for a series of nitrosoureas using Lewis-Lung screening data. The optimum lipophilic character for this series of compounds has been established. The optimum lipophilic character was determined previously for a similar series of nitrosoureas using L-1210 screening data. In comparing the results obtained for the two series of

nitrosoureas, the optimum lipophilic character was found to be approximately 1 to 1.5 log units higher using the Lewis Lung data. This would indicate that cancer cells in different locations in the body and/or different types of tumor cells are going to require agents with different partition coefficients to be most effective. Work in this area is continuing.

Some substituted 3,3-dimethyltriazenobenzenes have been prepared and submitted for testing. These have been prepared in order to explore the importance of the steric and electronic parameters for the triazeno-imidazole carboxamide series studied previously.

The comprehensive investigation of the parabolic structure-activity relationship in enzyme-substrate systems using α-chymotrypsin as a model has continued. The results obtained to date from the kinetic, ORD, and equilibrium binding studies are being evaluated and their implications assessed.

TABLE XIII

MISCELLANEOUS CONTRACT LABORATORIES

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Pomona College	Smith	NIH-NCI-C-73-3703

Program Review and Contractors Meetings

During the past year a comprehensive review of the contract synthesis program was made by a group of nine outstanding medicinal chemists. An in-depth review of the current synthetic program was presented to the Committee and each class of agents being prepared was discussed separately (see Branch report).

Two contractors meetings (Preparation Laboratory Contractors Meeting and Analytical-Radiolabel Contractors Meeting) were held during this report period. The major objective of these meetings was for the different contractors in a given area to discuss mutual problems, techniques and procedures. In addition, the Section Staff had an opportunity to review administrative procedures and to inform the contractors of changes in the Program. Consultants were present at both meetings and made presentations of interest to the group present. In addition, some of the contractors were requested to discuss topics of mutual interest to the group. All of the contractors present at both meetings felt the meetings were very helpful and express interest in holding additional such meetings. Additional meetings are being planned.

CHEMICAL AND DRUG RECORDS SECTION
DRUG DEVELOPMENT BRANCH

SCOPE

The responsibilities of the Chemical and Drug Records Section are (1) to acquire chemical compounds for the primary screen and for other program needs, (2) to develop, maintain and update systems of registry for all chemicals entering the program, (3) to maintain adequate storage and inventory of bulk chemicals and drugs, and (4) to coordinate the distribution of materials. These objectives are achieved through the concerted efforts by staff members to cultivate interest and to provide meaningful liaison with suppliers and potential suppliers; the surveillance of world-wide literature for new sources of chemical and drugs; the development of chemical nomenclature for all files; the distribution and utilization of chemical data; the monitoring of contracts; and the assurance that all discreet compounds obtained from commercial sources are maintained in accordance with the signed agreements.

STAFF

During this reporting period the Section was reorganized to accommodate the changes in DR&D program requirements. Mrs. Mary T. Flather was assigned to the Scientific Records Section, Program Analysis Branch, and Mrs. Nancita R. Lomax to the Chemical and Drug Procurement Section, DDB. The net effect of this reorganization is the reduction of two professional chemists from the Section and the transfer from the Section a major portion of the responsibilities for chemical nomenclature. The Section, however, still maintains, to a limited degree, the capability to assign systematic chemical names. Presently the professional staff consists of three chemists and one biologist. The supporting staff is made up of one secretary and two part-time clerk-typists.

IMPLEMENTATIONS

Acquisition of Compounds

Materials entering the screening program for the most part are voluntary contributions from research investigators at universities, government agencies, various non-profit institutions, industries and individuals interested in the area of chemotherapy. This continuous voluntary input of samples results partly from correspondence and personal visits of staff members to these various institutions.

To achieve the goal set for the increased number of synthetic materials to be tested in the primary screen, a compound collection contract was initiated in July, 1972 with Starks C.P., Inc. During the first six months the contract was in operation 13,900 compounds were collected and the precent of duplicates has been maintained at a very respectable level. Depending on the sources, the

percent duplication varies from ten to eighteen percent. The collection team has clearly demonstrated that through effective personal presentation it is possible to obtain the cooperation of industry, government laboratories and academic institutions. Starks C.P., Inc. has concentrated its collection efforts mainly in the United States and Canada and diligent liasion efforts by this group is providing for the collection of many compounds from hitherto new sources. The impact for the majority of the compounds collected by this team will not have its effect on the program until later.

To augment the Starks C.P., Inc. effort arrangements were made to purchase large blocks of chemicals from chemical supply companies such as Aldrich Chemical Company, Carbolabs, and Maybridge Chemical Company. The total number of purchased materials assigned new accession numbers was 2075.

Preliminary efforts have been made to develop new sources of materials from Europe and the Far East. Through the office of Dr. Omar Yoder, NCI Liaison Officer in Brussels, compounds are being delivered to NCI for evaluation. In addition a contract has been activated with Professor Silvio Garattini of Italy which will also result in a significant increase of compounds. Further increases will be realized when the Tokyo office becomes fully operational. Branch staff members have visited Hawaii, Fiji, New Zealand, Australia, Singapore, India and Canada to stimulate new suppliers to submit their products for testing.

During calendar year 1972, 51 universities were visited, 24 by the collection contractor and 27 by staff members. In addition, Starks C.P., Inc. collected samples from four government laboratories and eight industrial organizations. During this report period 291 new suppliers were added, of which 202 were new non-industrial suppliers. Among the active compounds 27 new ring systems have been identified, bringing the total to 291 active ring system. At the request of the Publication Section, Program Analysis Branch, the Section has checked the publication status of the compounds reported in 116 manuscripts submitted for publication clearance.

The following chart gives a breakdown by sources for the new compounds registered in 1972:

COMPOUND SOURCES	NUMBER	PERCENT
Non Industrial	7770	49.2
Industrial	4763	30.2
Purchased	2075	13.2
Contracts	1162	7.4
Total	15770	100.0

A fairly consistant level of 18% duplicates has been maintained even though there was an increase in the number of compounds entering the program. Under current

conditions about 18,880 compounds had to be processed to obtain 15770 new compounds for testing. Changes in requirements for the next calendar year have dictated that greater scrutiny in compound selection be emphasized. Guidlines to fulfill these requirements are under consideration. The new guidelines should also provide the mechanism to lower the percentage of duplicates.

Structure-Activity Studies

A recent version of the Hansch computerized regression analysis approach to structure-activity studies is available for use. An attempt was made to determine the structure-activity correlation for some compounds demonstrating activity in the 9KB system. Unfortunately results from this study did not indicate a good correlation. Currently, the Selected Agents List is being reviewed in order to select appropriate candidates for structure-activity studies. In addition to the Hansch approach, other methods to structure-activity correlations, such as the Free-Wilson approach, are being evaluated.

Chemical Information

The Section cooperated with the Program Analysis Branch in the registering of the total chemical file for computerized information retrieval. All new submission are registered and duplicate checks are made automatically. Significant support has also been provided by Staff members in the development of the new chemical information system under contractual arrangement with the University of Pennsylvania. For rapid retrieval of chemical information Master Chemistry Card Files and Molecular formula files are maintained. During this report period the Section processed over 4,400 new submissions for duplicate search and registration at Chemical Abstract Services. In addition, the Section continues to process manually those compounds which require priority testing.

In compliance with the order issued by the Food and Drug Administration, the Section monitors and maintains records of input and distribution of materials considered by FDA as narcotics, barbiturates, hallucinogenic and other dangerous drugs. The FDA has specified that separate and accurate records be maintained for these materials and only these individuals with the proper narcotic license be approved to dispense and receive these items. To insure proper safety, the drug abuse compounds are secured in a separate file under lock and key.

The confidential status of compounds obtained from industrial sources requires constant up-dating and careful monitoring. The recognition by these organizations that the Branch maintains constant surveillance over commercial discreet materials has afforded not only a good industrial relationship but has made available many commercial sample not otherwise accessible. Frequently, there is a need to obtain the release of compounds from confidential status. Declassification is achieved only when the industrial supplier agrees, in writing.

CHEMICAL AND DRUG DISTRIBUTION

The Chemical and Drug Records Section is also responsible for the receiving, storage and distribution of chemicals for screening. The Section is charged with the custody of bulk chemicals and drugs that enter the Program. Detailed inventory records, such as source, date received, amount, lot number, initiator of all shipments and destination of drugs are recorded on a daily basis.

The Section monitors the portion of the contract with Microbiological Associates, Inc., (PH43-NCI-C-68-1283) which handles the receiving, weighing, record-keeping, distribution and storage of compounds. Over 160,000 different materials are held in storage. These consist largely of chemicals and to a lesser extent plant products, antibiotic and steroids. A total of 12,995 new compounds were shipped to the screening laboratories; 10,464 refills and materials for special testing were weighed and 2583 packages were shipped. During 1972 approximately 18,740 samples were returned by the screening laboratories. These compounds, for which testing has been completed, are entered into the inventory.

New Areas Under Development

With changing program requirements the Section is considering the development of protocols that will provide guidelines for the selection of compounds, is investigating improved methods of reporting information to suppliers, is searching for better containers that will meet the requirements of the screening laboratories and is improving methods for literature surveilance.

During the past year the Section has participated in the monitoring of the contracts listed in Table VII.

TABLE VII

CONTRACTS MONITORED BY THE SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Chemical Abstracts Services*	Brown	PH-43-NCI-C-65-1021
Microbiological Associates, Inc.	Berman	PH-43-NCI-C-68-1283
University of Pennsylvania*	Hill	NIH-NCI-C-71-2187
Starks C. P. Inc.	Starks	NIH-NCI-C-72-3705

*Contracts with Program Analysis Branch

CLINICAL DRUG DISTRIBUTION SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is primarily responsible for the development, production, control and distribution of all investigational dosage forms of interest to the Division of Cancer Treatment Program. The Section is also responsible for the procurement and distribution of commercial formulations used by the Clinical Cooperative Groups.

The responsibilities of the Section can be categorized as follows:

1. Development of investigational formulations for clinical use;
2. Production and packaging of investigational drugs;
3. Purchase of formulations for clinical use;
4. Quality control surveillance of all clinical dosage forms;
5. Preparation of pharmaceutical data for FDA-IND submissions; and
6. Storage and distribution of clinical dosage forms.

Staff

The staffing of this Section consists of three Pharmacists, one NIH Visiting Fellow, three shipping and receiving clerks and one secretary.

Development of New Dosage Forms for Clinical Use

Formulation development commences when an agent passes Decision Network IIA. The first approach to development involves an evaluation of existing chemical and physical data and a review of in vivo route and regimen studies in rodents. The accumulation and evaluation of these data are critical to the ultimate design of the dosage.

Developmental studies are assigned by this Section to one of the contract pharmaceutical laboratories. In preparing the assignment, the contractor is provided with the route, dose level, and supporting available chemical data. Consultation with the Project Officer is carried out concerning feasible formulation approaches.

Routinely, agents considered for parenteral formulation are subjected to solubility and stability studies in a selected group of physiologically accepted vehicles. The drug solutions are then studied for the effects of pH, temperature, light and atmospheric oxygen. Additionally, compatibility tests are performed with buffers, preservatives and vial closures. Agents considered for oral formulation are studied for compression properties, compatibility with binders, photodegradation and temperature effects.

Some of the drugs recommended for development are either insoluble or poorly soluble in most commonly used physiological vehicles. Occasionally, these compounds are highly reactive and require special handling. These problems have been an obstacle in the preparation of some parenteral dosage forms of potential antitumor agents. The contract with the University of Kansas is exploring these formulation difficulties. The contract is designed to perform research on current formulation problems and to design new approaches to parenteral drug delivery.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses.

The toxicological results and the accumulated pharmaceutical development data are distributed to members of the Dose Formulation Committee. These data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the development limits of the dosage form.

During 1972, 17 new dosage forms were developed and 11 of these entered clinical trial. An additional 18 dosage forms were modified from existing preparations.

Production and Packaging of Investigational Drugs

An important function of the Clinical Drug Distribution Section is to provide the Clinical Program with sufficient quantities of formulated materials. The production of dosage forms is mainly carried out by contract agreement with qualified pharmaceutical manufacturers.

During 1972 the production of clinical products increased substantially. The production of oral solid dosage forms (tablets and capsules) increased by 172% and parenteral product production increased by 64%. The formulation contractors produced 26,703 bottles of tablets or capsules and 504,626 vials. Production output which is dictated by clinical needs has continued to increase during the last few years. The production contractors have been able to meet the Program's needs for formulated materials. Four production contracts were monitored by this Section: Ben Venue Laboratories, Philips Roxane Laboratories, Warner-Lambert Research Institute, and The University of Tennessee.

TABLE XVI
CONTRACTS MONITORED BY THE CLINICAL DRUG DISTRIBUTION SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ben Venue Laboratories, Inc.	Wickes	PH43-NCI-C-66-909
Kansas, University of	Higuchi	NIH-NCI-C-72-3217
Philips Roxane Laboratories	Ebert	NIH-NCI-C-71-2139
Tennessee, University of	Avis	NIH-NCI-C-70-2084
Warner-Lambert Research Inst.	Fand	NIH-NCI-C-72-3250

Some contractors primarily engaged in bulk drug preparation have capabilities for dosage form production. This Section encourages these contractors to develop and produce their own dosage forms. Specifications for formulation and production are provided to each contractor by this Section. This arrangement has proven economical, expedient and extremely beneficial to the program. Two contractors, The Upjohn Company (Contract No. PH43-NCI-C-68-1023) and Bristol Laboratories (Contract No. PH43-NCI-C-69-35), have provided formulated materials by this arrangement during 1972.

Several dosage forms are developed and produced by pharmaceutical manufacturers in cooperation with the Division of Cancer Treatment. By such an agreement the manufacturer supplies the dosage form free of charge or on a cost sharing basis in return for toxicological and/or clinical data. These sources include several foreign drug manufacturers. The dosage forms are prepared from specifications which are established by mutual agreement between this Section and the manufacturer. During 1972, the following companies supplied a total of 138,357 vials and 75 bottles of tablets and/or capsules through this joint research effort:

Bristol Laboratories, USA	NSC-125066	Bleomycin
Eli Lilly & Company, USA	NSC-403169	Acronycin
E. R. Squibb and Sons, USA	NSC-23759	Testolactone
Farmitalia, ITALY	NSC-123127	Adriamycin
Ives Laboratories, USA	NSC-82151	Daunorubicine
Kojin Company, JAPAN	NSC-145668	Cyclocytidine
Mead Johnson Laboratories, USA	NSC-109724	Ifosfamide
Merck, Sharp and Dohme, USA	NSC-109229	Asparaginase
Merrell National Laboratories, USA	NSC-151466	cis-Clomiphene
Microbiological Research, ENGLAND	NSC-106977	Erwinia Asparaginase
Parke-Davis & Co., USA	NSC-404241	Adenine Arabinoside
Pasteur Institute, FRANCE	NSC-B116117	BCG Vaccine
Spofa, CZECHOSLOVAKIA	NSC-104801	Cytembena

Purchase of Dosage Forms

Several investigational dosage forms are procured by direct purchase from pharmaceutical suppliers. The product specifications are prepared by the Section and the final product is analytically tested by an independent laboratory and reviewed by the Quality Control Committee for Chemicals and Drugs before release for clinical use. During 1972, 11 investigational dosage forms were purchased involving 1,724 bottles of tablets or capsules and 99,469 vials.

This Section also obtains commercially available dosage forms for distribution to the Clinical Cooperative Groups. These dosage forms are purchased either through the Veterans Administration Supply Contracts, or by special purchase through negotiated contract mechanism. All suppliers of commercial formulations are required to supply a certificate of analysis on each lot of drug purchased. The data is reviewed for compliance with strict manufacturing criteria. A total of 200,752 vials and 24,752 bottles of tablets and/or capsules were purchased during calendar year 1972.

Table XVII summarizes the procurement activities of the Section during calendar year 1972.

TABLE XVII
DRUG ITEMS PROCURED

SOURCE	Capsules/Tablets 1971	Capsules/Tablets 1972	Vials 1971	Vials 1972
Produced by Contractors	694,161	1,704,500	306,506	504,626
Contributed or Cost Sharing	155,520	7,500	110,574	138,357
Purchased Invest. Drugs	235,000	152,400	40,827	99,469
Purchased Commercial Drugs	1,721,400	2,666,600	151,405	200,752
	2,806,081	4,531,000	609,312	943,204

1971 TOTAL: 3,415,383

1972 TOTAL: 5,474,204 (62% increase)

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must meet specific quality control criteria prior to release for clinical use. This Section, in collaboration with the Chemical and Drug Procurement Section, maintains a strict quality control program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., and oral products are tested for content uniformity, weight variation, hardness, disintegration, etc. All products are tested for potency by the formulator and sometimes by another laboratory.

Individual unit assays are required; the number of samples assayed depend on the dosage form and the size of the production batch. All new dosage forms are routinely submitted to an outside analytical contractor to verify the analytical methodology and accuracy of the formulator. The confirmation analyses are mainly performed by the contract analytical laboratories, Stanford Research Institute and Midwest Research Institute.

This Section participates in the activities of the Quality Control Committee for Chemicals and Drugs. This committee reviews for clearance the analytical results of bulk chemicals and formulated clinical dosage forms. During calendar year 1972, 263 lots of clinical dosage forms were reviewed by the Quality Control Committee for Chemicals and Drugs. Of these, 154 were investigational dosage forms and 109 were commercial dosage forms.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of the investigational dosage form. These studies are carried out under simulated clinical conditions to test the effects of temperature, light and salt additives. The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

This Section also maintains a shelf-life surveillance program on all lots of non-commercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Two assay schedules are used for shelf-life studies: Schedule A, designed to provide stability data under accelerated conditions; and Schedule B, designed for controlled studies of repeat formulations were adequate stability data is available. Both schedules provide a two-year surveillance record.

Preparation of Pharmaceutical Data for FDA-IND Submissions

This Section prepares attachments I, II, III, V and VII for submission to Cancer Therapy Evaluation Branch for IND filing with the Food and Drug Administration. These attachments consist of the description composition, method of manufacture and controls used in the preparation of the dosage form. The FDA requires that such a submission be made for each new investigational drug prior to clinical release. In addition, IND amendments must be submitted whenever a new dosage form of an existing IND drug is formulated for clinical use.

During 1972, this Section prepared 4 original IND reports and 14 amended IND reports.

Storage and Distribution of Clinical Dosage Forms

The receiving and shipping activities of the Section are carried out by three shipping clerks. These clerks have been instructed in the required handling techniques for each drug. They are knowledgeable of postal and freight procedures used in this country and throughout the world. A significant amount of clinical drugs are shipped to foreign investigators each year. During 1972, approximately 665 foreign shipments were made. During the same period 9089 domestic drug shipments were made.

During 1972, a total of 935,353 drug items (vials and bottles) were received and inventoried for subsequent world-wide distribution. During that same period, 560,265 drug items were distributed (30% increase over 1971) averaging 39 individual shipments daily.

This Section is also responsible for coding of drugs for double-blind studies. These studies may involve two or more drugs; the same drug in different dosage strengths, or a combination thereof. In all instances, these dosage forms are indistinguishable in appearance for double-blind purposes. The codes are placed on each bottle or vial by a supply clerk and checked by the pharmacist prior to shipment. There are presently 19 such coded studies on inventory.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. Returned drug information is recorded on the original inventory file, including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping date is also on file by investigator's name, quantity shipped, etc. This Section is responsible for the disposition of the returned drug. The judgement to

restock, destroy or quarantine a returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors.

During 1972, the Section handled an average inventory of 408 dosage forms by lot number involving 204 different formulations of 135 different compounds. These figures include investigational and commercial dosage forms.

Serial No.: 3584
1. Division of Cancer Treatment, DR&D
2. Drug Development Branch
3. Clinical Drug Distribution Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Research in Dosage Form Development of New Antitumor Drugs
Previous Serial Number: 3584
Principal Investigator: J. Paul Davignon

Other Investigators: Kim W. Yang, James C. Cradock, John S. Driscoll and Harry B. Wood, Jr.

Man Years

Total: 1.25
Professional: 1.25

General Objectives:

The objectives of this project are to:

(1) Study new formulation approaches with potential utility for those compounds posing difficult formulation problems.

(2) Modify existing parenteral formulations to yield preparations more physiologically compatible with cerebrospinal fluid.

(3) Evaluate dosage form problems presented by clinical investigators.

Specific Objectives:

(1) Development of a parenteral dosage form for NSC-95441 (Methyl CCNU) and for NSC-79037 (CCNU).

(2) Modification of the available parenteral form of NSC-77215 (Procarbazine) for toxicological evaluation by the intrathecal route of administration.

(3) Formulation of a more suitable parenteral dosage form of NSC-71795 (Ellipticine).

(4) Evaluation of the effects of a non-ionic surfactant on solubility of certain poorly water soluble antitumor agents.

Major Findings:

I. Development of Parenteral Dosage Forms of Methyl CCNU and CCNU.

Although Methyl CCNU and CCNU readily dissolve in ethanol, subsequent dilution with aqueous solutions to 5-10% ethanol concentration was accompanied by rapid precipitation of either nitrosourea. However, preparations containing Methyl CCNU or CCNU as an equal volume mixture of ethanol and the non-ionic surfactant, polyethoxylated vegetable oil (PEVO), yielded clear solutions after dilution with 9 volumes of water or commonly used intravenous fluids. The solubilities of Methyl CCNU (2.5 mg/ml) and CCNU (1.5 mg/ml) appear adequate for administration in about 150 ml of ethanol-PEVO-intravenous fluid, 5:5:90 as a slow (1/2 - 1 1/2 hour) intravenous infusion.

A pyrolysis gas chromatography procedure was developed and employed to assess the stability of Methyl CCNU and of CCNU in ethanol-PEVO-water, 5:5:90. Less than 5% decomposition was noted in either preparation after six hours exposure to room temperature and light.

The ethanol-PEVO-5% Dextrose in water formulations of Methyl CCNU and CCNU were evaluated in vivo for antitumor efficacy in the L1210 system. These preparations were administered i.v. and i.p. and the activities were found to be $\geq$ to the respective nitrosourea administered in suspension. These formulations are presently undergoing toxicological evaluation in dogs and monkeys.

II. Design of Dosage Forms for Intrathecal (I.T.) Administration.

Drug formulations intended for intravenous administration frequently contain additional agents to enhance stability or solubility. Due to rapid dilution and buffer capacity of blood, these additives usually are pharmacologically inoccuous. However, these same formulations may not necessarily be administered intrathecally with the same margin of safety due to cerebrospinal fluid (CSF) volume or CSF buffer capacity. Parenteral Procarbazine is formulated as the hydrochloride salt and aqueous solutions exhibit a pH of about 2.9. This preparation also contains antibacterial preservatives and a chelating agent. A formulation of Procarbazine without additives and exhibiting a pH of 6-8 was desired for intrathecal administration. Formulations of this compound at pH 6-7 were soluble but quite susceptible to oxidation. A compounding procedure was developed that provided for preparation of Procarbazine 200 mg/vial. Pertinent aspects included compounding and dispensing under a nitrogen atmosphere, lyophilization and sealing vials under vacuum. This formulation yielded clear solutions (pH 6.5 - 7.5) upon reconstitution, did not contain additives, and was as active as the HCl preparation versus L1210 in vivo. This new preparation has been submitted for toxicological evaluation.

III. Formulation of NSC-71795 (Ellipticine)

Ellipticine as the HCl salt was noted to be hemolytic in vivo and in vitro. Addition of citrate buffer pH 3.3 abated hemolysis, however. Citrated formulations of ellipticine and NSC-69187, 9-methoxyellipticine, were desired for evaluation of treatment schedule dependency vs L1210. A preparation containing both ellipticine and citrate buffer in the same vial was not feasible. Instead ellipticine was formulated as the HCl salt and supplied as a lyophilized dosage form. The citrate buffer was packaged separately and used to reconstitute ellipticine. 9-Methoxy-ellipticine was formulated in the same manner.

IV. Solubilization of Certain Antitumor Agents Using a Non-ionic Surfactant

The effect of the non-ionic surfactant, polyethoxylated vegetable oil (PEVO), on solubilization of the nitrosoureas, Methyl CCNU and CCNU, led to the further evaluation of this vehicle on other poorly water soluble agents. The compounds evaluated included: NSC-403169 (acronycin), NSC-141549 (methanesulfon-m-anisidide,4'-(9-acridinyl-amino)-, monohydrochloride), NSC-145669-(2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinone), NSC-143414 (benzyl dimethyl rifampicin) and NSC-13875 (hexamethylmelamine).

Solutions of NSC-403169 were prepared in ethanol-PEVO-physiological saline, 5:5:90 and in dimethylacetamide (DMA)-PEVO-physiological saline, 5:5:90. Both preparations represent a 200 to 400 fold increase in acronycin water solubility. NSC-141549 is readily soluble in DMA but is poorly water soluble (~0.25 mg/ml). Clear solutions of NSC-141549, 2.5 mg/ml, were prepared in DMA-PEVO-physiological saline, 5:5:90. Similar improvements in the water solubility of NSC-143414, NSC-145669, and NSC-13875 were noted using the DMA-PEVO-physiological saline, 5:5:90 system. These preparations will be evaluated in the appropriate tumor systems. PEVO appears to provide a formulation approach for certain compounds that are ethanol or DMA soluble but poorly water soluble.

V. Miscellaneous Projects

The availability of a dosage form development laboratory has enabled a number of small projects to be carried out in cooperation with other groups within the Division of Cancer Treatment. Fourteen small batches of parenteral products were prepared. These materials included lyophilized and liquid fill preparations and were subsequently evaluated for pharmacological and/or toxicological effects.

NATURAL PRODUCTS SECTION
DRUG DEVELOPMENT BRANCH

Scope

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from microbial sources, higher plants and animal products. This area will be discussed under the headings of Fermentation Products Program and Plant and Animal Products Program.

Staff

This Section has operated with a staff of four persons, consisting of two professional (Ph.D. level) and two clerical employees. Dr. Alfred Stanley, who was responsible for the fermentation products program, retired on June 16, 1972; he was replaced by Dr. John Douros on November 27, 1972.

Surveillance

World-wide surveillance of the published literature and collection of chemicals and drugs for biological evaluation are carried out in the Fermentation Products area. With the dropping of the plant literature survey project by the Program Analysis Branch and the inability to assign additional personnel to this work, it has not been possible to undertake surveillance in the Plant and Animal Products area.

Statistical Summary

Table I lists the natural product materials acquired during the year under review compared with the previous year. Of the crude extracts, contractors provided all of the 2712 fermentation products, 76% of the 1669 animal products, and 89% of the 10,434 plant products. A total of 136 purified (mostly crystalline) natural products were received. Nineteen compounds passed the criteria for confirmed activity (Step III). Five new compounds from natural sources were assigned to Decision Network IIA, one being a chemical modification of a fermentation product. Four compounds were assigned IND numbers, of which one was a fermentation product, one was a plant product and two were chemically modified plant products.

FERMENTATION PRODUCTS PROGRAM

Since the initiation of antibiotic research and fermentation in the early 1940's, microorganisms have been an extremely important source of new chemotherapeutic agents in the infectious disease field and 37 have been evaluated in U.S. clinical studies for antineoplastic activity. Antibiotics have a great potential in cancer chemotherapy as primary treatment agents or as the initial structural building blocks for new and novel compounds that can be synthesized and used in the clinic.

Three groups of organisms have been shown to be the principal producers of antibiotics; they are bacteria, fungi, and actinomycetes. There are still multitudes of new and different organisms to be isolated, the extent of

which is limited only by the ingenuity and skill of the microbiologist. For instance, various substrates other than glucose and other sugars (terpenes, purines, aromatics, olefins, known antibiotics, natural products, fatty acids, etc.) can and should be introduced as carbon sources which will cause the organisms to produce different metabolic products. Microbiological transformations and co-oxidation fermentations are different techniques to produce varied products and unique antibiotics.

No single family of organisms has been thoroughly investigated for what it can produce by fermentation using a varied number of carbon sources and co-oxidation. Microbial transformations of the known toxic, active and inactive antibiotics and natural products have not been undertaken in order to see if microbial modifications can make them of clinical value. Yeast, thermophilic and psychrophilic microbes, and mixed cultures are other possibilities which might be investigated for the possibility of producing antineoplastic compounds.

Review of the literature to obtain worthwhile new antibiotics continues. As a result of this surveillance, 41 partially purified and 27 purified materials were obtained from outside sources. One additional purified antibiotic was received from our contractors. Thirty-eight antibiotics have been in clinical study and up to the present 11 are still under clinical investigation. Those still under clinical study are L-asparaginase from Escherichia coli, L-asparaginase from Erwinia carotovora, Bleomycin, Azaserine, Mitomycin C, Porfiromycin, Streptonigrin, Streptozotocin, Chromomycin A_3, Adriamycin, and Daunomycin. Several antibiotics are being clinically evaluated in other parts of the world. They are Neocarzinostatin, Olivomycin A, Anguidine, and Actinomycin C. All but Actinomycin C are under development in our program.

The present fermentation program has been carried out through contracts at the different biological laboratories listed in Table XIV.

Table XIV

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Bradner	NIH-NCI-C-69-35
Illinois, University of	Rinehart	NIH-NCI-C-72-3208
Upjohn Company	Neil	NIH-NCI-C-73-3707

Each contractor has been using its own approach to soil collections, culture isolation, selection of culture types, fermentation development, and chemical isolation. The reduction in number of contracts, during the past several years, and the diluting of effort in fermentation in the present contracts have had a marked effect on the number of broths being fractionated and on the number of purified samples received for pre-clinical evaluation. Twenty-two high priority culture fermentations are under fractionation and it is expected that many will be purified and evaluated in the coming year. Additional antibiotics should also be obtained from our surveillance program and evaluated.

The Upjohn contract has used a cytoxicity test as their initial screen and then ranked their leads according to cell cycle specificity. The cytotoxic actives, when tested in vivo, had approximately a 40% active response. Emphasis is still primarily to evaluate fungi in fermentations. This year a more diverse approach to culture isolation, selection and fermentation is being discussed and may be implemented.

One agent has passed DN II--4-Benzyl-2,6-dimethyl-rifamycin (NSC-143,414), a chemically modified rifamycin. An IND has been filed for one agent--Chromomycin A_3 (NSC-58,414). Neocarzinostatin (NSC-69,856) and Anguidine (NSC-141,537) are in toxicology.

PLANT AND ANIMAL PRODUCTS PROGRAM

In carrying out its objectives in the plant and animal products area the Section has, in addition to the contract effort, developed programs with collaborating research grantees and other organizations in (a) plant and animal collecting; (b) preparation of extracts; (c) fractionation of active extracts, and the isolation and identification of the active agents therefrom; and (d) in collaboration with the Chemical and Drug Procurement Section, the preparation of large amounts of active agents from plant materials for preclinical toxicology and clinical trial.

Through extensive correspondence, contact is maintained with potential domestic and foreign suppliers in order to acquire plants, animals, and pure compounds of natural origin which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular, native, and folklore).

The year under review is the second year benefiting from the increased funds provided toward the end of FY 71 and is the year in which the goal of increased input into the program has been achieved. Because of the increasing necessity in recent years to re-collect large amounts of natural products for fractionation, and the reduction in dollar amount of the contracts, the general collection of new plants under contract had substantially decreased from a normal level of approximately 5000 per year to about 3000. This has had an adverse effect on the number of pure compounds coming out of the program. Other factors causing a drop in the number of pure compounds are (a) the discontinuance of fractionation work during and since FY 1971 on plants active in Walker 256 (WM) and other tumors, and (b) the substantial backlogs at the screening contractors until the fall of 1971. During the year under review 10,434 new crude plant extracts and 1669 new crude animal extracts were accessioned. This should result eventually in an increased number of DN II compounds of novel chemical structure and of drugs for clinical trial.

The following contractors are engaged in the Plant and Animal Products Program (Table XV).

In addition, open-end, fixed-price contracts for the procurement of plant extracts have been put into effect with Northeastern University (Dr. R. F. Raffauf) and with the University of Costa Rica (Dr. J. Saenz R.).

Table XV

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Agriculture U.S. Department of	Perdue	NCI-CR-64-1 (Transfer)
Arizona, University of	Cole	PH43-NCI-C-67-1484
Arizona State University	Pettit	NIH-NCI-C-71-2308
Central Drug Res. Inst.	Dhar	01-004-1 (PL-480)
Hawaii, University of	Sagawa	PH43-NCI-C-67-748
Illinois, University of	Farnsworth	NIH-NCI-C-72-2078
Research Triangle Inst.	Wall	NIH-NCI-C-69-2019
Virginia, University of	Kupchan	PH43-NCI-C-64-551
WARF Institute, Inc.	Derse	PH43-NCI-C-67-747

Beside the contractors listed above, several groups, including grantees and those not supported by the NIH, contribute in different ways to this program without cost to DR&D. The major groups are: C.S.I.R.O. (Australia), U.S.D.A. (Pesticide Chemical Research Branch, Beltsville), U.S.D.A. (NURDD, Peoria), C.S.I.R. (South Africa), National Defense Medical Center (Taiwan), Purdue University (Dr. Cassady), Virginia Polytechnic Institute (Dr. Kingston), Univ. de Concepcion, Chile (Dr. Silva), and Univ. of Oklahoma (Dr. Weinheimer).

Progress was made in the following areas of natural products:

A. Marine organisms

During the year, 1669 animal products, mostly marine, were accessioned. About 84 different species are active in Leukemia P388 (PS) and about 30 species are currently under fractionation. It is anticipated that pure products will be forthcoming from this work in the near future.

B. Plants

1. General collections. The current rate of collecting has been increased to about 10,000 per year. The U.S.D.A. has increased its general collecting to 8000 per year; the University of Hawaii has increased its general collecting to a rate of over 500 per year; and the University of Costa Rica has doubled its collecting to a rate of over 400 per year. The remaining extracts come from miscellaneous sources.

   Improvements have been made in our plant collection procedures which will ultimately increase the yield of active agents and shorten the time lag in obtaining active agents. Continuing analyses have shown that certain families and genera yield a relatively higher proportion of active agents, while others yield a low proportion. Where possible, more intensive collections of "rich" families and genera are carried out, while the "poor" families are avoided.

2. Large Re-collections. These are of two kinds: 50-100 lbs. for fractionation work (150-250 lbs. where possible) and 1000-30,000 lbs. for isolation of large quantities of active drugs needed for pre-clinical toxicology and clinical trial. The former have been increased in number to over 200. Five very large re-collections were made during this report period. These are: Maytenus buchananii (for maytansine, NSC-153858), 20,000 lbs. from East Africa; Fagara macrophylla (for nitidine, NSC-146396, and methoxydihydronitidine, NSC-146397), 8000 lbs. from Ghana; Heliotropium indicum (for indicine N-oxide, NSC-132319), 11,700 lbs. from Taiwan and Brazil; Tripterygium wilfordii (for triptolide, NSC-163062, and tripdiolide, NSC-163063), 2500 lbs. from Taiwan; and Cephalotaxus harringtonia (for harringtonine, NSC-124147, and homoharringtonine, NSC-141633), 1300 lbs. from Virginia.

   In addition, steps have been taken to increase the supply of the plant Cephalotaxus harringtonia (for harringtonine and homoharringtonine) by establishing large-scale cuttings at the U.S.D.A. Plant Introduction

Station in Georgia and at different nurseries. And measures have been taken to increase the supply of Thalictrum dasycarpum (for thalicarpine, NSC-68075) by obtaining and analyzing different strains of seeds, and conducting experiments on best methods of growing the high-alkaloid strains. Both the Cephalotaxus and Thalictrum are not plentiful enough in their native state to be adequate sources of their respective active agents.

3. Duplicate Searching: Automated procedures have been developed with the Program Analysis Branch which are making possible a more efficient and economical accessioning and testing of plant and animal materials. This concerns the search for "duplicates" from a list of names of confirmed active plants and of those inactive plants of which we have had ten or more samples, which we do not wish to accession again for testing. These samples include different parts of the plants, samples from different geographical locations or collections at different times of the year, and extracts prepared with different solvents. A frequency distribution of the number of collections of each plant name, prepared by computer, showed a rough cut-off point at 10, at which point it appeared that 97 per cent of the plants were collected 10 or fewer times. A 3% loss to duplication seemed acceptable, especially since, using a manual search of a card file, only about 2000 names a year could be searched. With this improvement the present input of about 10,000 names per year, and any foreseeable increase, can be handled readily. The saving in extraction and screening should be substantial.

4. Increasing the yield of Leukemia L-1210 (LE) actives: Following discussions at the November 1971 Annapolis contractors' meeting and decisions made thereafter, it has been possible to implement a recommendation directed toward increasing the yield of LE actives by subjecting a number of plant materials to a procedure designed to concentrate any LE-activity present to a level where it can be detected by the regular screen. This procedure is a preliminary fractionation by solvent partition yielding five extracts from every plant material. Results from this test should be available within the next year.

5. Decision Network IIA and IND Materials: Four plant-derived compounds passed DN IIA--homoharringtonine (NSC-141,633), maytansine (NSC-153,858), triptolide (NSC-163,062) and tripdiolide (NSC-163,063).

   IND's were filed on three plant-derived compounds--thalicarpine NSC-68075), epipodophyllotoxin, 4'-demethyl-,9-(4,6-O-2-thenylidene-β-D-glucopyranoside) (NSC-122,819) and epipodophyllotoxin, 4'-demethyl-4,6-O-ethylidene-β-D-glucopyranoside (NSC-141,540). Of these, the latter two are simple chemical modifications starting with naturally-occurring compounds.

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ANNUAL REPORT

DRUG EVALUATION BRANCH

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

Principal objectives of the Drug Evaluation Branch (DEB) are to initiate, develop, and coordinate laboratory programs for recognition, recommendation, and development of new drug therapies against human cancer. Objectives are achieved by (a) screening large numbers of synthetic and natural products for biological activities suggesting potential clinical usefulness, (b) evaluating "active" drugs with respect to conditions of usage (e.g., treatment route and schedule, combined modalities of treatment) which may enhance therapeutic efficacy leading to cures of animal tumors, (c) determining drug effects on host immune responses and their implications to therapy, (d) studying experimental means of altering antigenicity of tumor cells and thus their sensitivity to chemotherapy, (e) investigating mechanisms of drug action and resistance, (f) developing new and improved methods for drug evaluation, and (g) investigating related specific problems arising during preclinical toxicological examination, clinical formulation development, and clinical trial. Studies are aimed at providing information essential to achieving objectives as defined in the DCT Program's Linear Array, at research contributing directly to the development of methods for providing such information, and basic research of significance to understanding relevant scientific principles that could have a major impact on program objectives. Program is implemented through contracts with qualified institutions and through intramural laboratory projects.

DEB includes the Office of the Chief (J. M. Venditti) and three sections: Biochemistry (F. R. White, Head), Immunotherapy and Immune Response Modifiers (IIRM; A. Goldin, Office of the Associate Scientific Director, DR&D, Principal Advisor), and Screening (B. J. Abbott, Head); and is staffed by 11 senior professionals (including 2 staff fellows, 1 staff associate, and 2 commissioned officers) and 16 junior professional, technical and clerical employees.

Staff have primary responsibility for 19 contracts plus two PL-480 agreements, and responsibility for drug evaluation related activities carried out under six contracts and two PL-480 agreements administered by other DCT units. Negotiated or obligated funds for DEB monitored contract activities totaled approximately $9,400,000/a. as of January 31, 1973. The relationship of DEB-monitored contract and intramural projects to activities outlined in the DCT Linear, Supplementary and Exploratory Arrays are shown in Tables 1-4. During calendar year (CY) 1972, 30,585* synthetic and natural products were submitted

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*All references to materials submitted in this report were taken from the PRA report of January 23, 1973 "Materials Added to the Master Files".

to DR&D for primary screening. This was approximately twice the number submitted in CY 1971. During 1971 and early 1972, in vivo screening capacity was progressively increased in anticipation of the marked increase in input of materials. Thus during CY 1972, 303,105 in vivo primary screening tests were conducted (103,681 against mouse leukemia L1210; 180,027 against mouse leukemia P388; and 19,397 against other transplanted rodent tumors). Excepting synthesis contractor-generated congeners of known active materials, synthetic agents are screened initially against L1210 and crude natural products are screened initially against P388. All materials are tested in cell culture. Cell culture testing of crude natural products provides a relatively inexpensive tool for bioassay during fractionation of those with both in vivo and in vitro activity. Regardless of the assay used for bioassay, purified natural products are tested against L1210. Routine cell culture testing of randomly acquired synthetics is aimed at the selection of highly cytotoxic materials that are not active against L1210 so that they might be studied in more detail against other selected experimental tumors. In addition, these may, on a selective basis, be tested for their effects on specific biochemical sites; thus providing a potential basis for discovering new important biochemical pathways that might be exploited for drug design. The basis for current screening practice has been presented previously in these reports and the reports of the annual DCT Contractor's Workshops. Substantially current protocols used for primary screening were published during the last year (see Geran, et al, "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems", third edition, Cancer Chemotherapy Reports, Part 3 3: 1-103, 1972). At present, only the L1210 and P388 systems are being used routinely. Compounds that pass the DCT-Decision Network (DN) Point 2A and other selected compounds are screened against B16 melanoma and Lewis lung (LL) carcinoma in mice to provide additional information relative to potential usefulness against human tumors. A more limited number of drugs are tested against AKR lymphoma and C3H mammary carcinoma. A recent analysis of comparative activity against human solid tumors, L1210, P388, B16 and LL suggested that L1210 and P388 used for initial screening of synthetics and natural products, respectively and subsequent testing against LL and B16 would have identified most of the agents of known activity against human solid tumors (Table 5). These observations are of importance relevant to the following report on the current structure of the in vivo primary screening program and our recommendations for its modification. The primary screen and other DEB-related projects are listed in Tables 1-3 and are coded appropriately. The cost figures used (Tables 1-3) were based on funds obligated in FY '72 or actually negotiated as of January 31, 1973. In only one case (Southern Research, Contract-29) was an estimate for FY '73 used. It should also be noted that the costs shown relate to DEB-monitored projects only and do not include animal or data processing costs.

The total funding for DEB-related tasks overall (approximately $9,400,000) is being utilized approximately as follows:

Bioassay of natural products in vitro (L-1XX)	3.0%
Bioassay of natural products in vivo (L-1XX)	7.0%
Other Bioassay-related tasks (L-1XX and E-1XX)	1.3%

Screening - in vitro (L-2AX)	3.5%
Screening - in vivo - L1210, P388 (L-2AX)	35.1%
Screening - in vivo - Other tumors (S-2AX)	1.5%
New Assay development - Screening in vitro (E-2AX)	0.5%
New Assay development - Screening in vivo (E-2AX)	10.2%
(1st generation AKR lymphoma)	(1.3%)
(B16 & LL Models, Mini-screens, etc.)	(7.6%)
(In vivo tumor cell kinetics)	(1.3%)
New Assay Development - Biochemical (E-2AX)	0.4%
Detailed Drug Evaluation - in vivo - established tumor systems (L-2B2)	15.1%
Detailed Drug Evaluation - in vivo - special assays; spontaneous AKR, immunology, etc. (S-2B2)	6.9%
Detailed Drug Evaluation - in vitro - (S-2B2)	2.2%
Detailed Drug Evaluation - Biochemical Mech. (S-2B2)	3.5%
Detailed Drug Evaluation - in vitro - development (E-2B2)	0.7%
Development - Animal Models of Human Tumors (E-2B2)	8.6%
Tumor Bank	0.5%
	100%

I. Initial Screening of Crude Plant and Animal Products and Bioassay of Fractions.

We can estimate the cost of screening of crude plant and animal materials at about $1,500,000/a. (40% of L-2AX) and the cost of bioassay of fractions at about $500,000/a. (60% of L-1XX). These estimates do not include the costs of plant collection and recollection or the extraction and fractionation work of plant chemists. Some effort to increase cost efficiency in this area would be worthwhile, and a modification of operational procedures to select materials with the greatest potential for overall clinical utility prior to recollection would seem appropriate. It is recommended that plant and animal products be handled in the following general way.

Stage I, Step 1. Screen crude materials against IP-P388 using an economical modification of the "mini-screen" approach (see this report, Part IIIA), e.g., 3-4 mice/gp. rather than 6, one treatment schedule rather than two. The reduction in number of animals per test group is more hazardous for crude natural products than for synthetics because of the nature of the materials tested but it should be tried. Set criteria for activity at this step rather low (T/C% $\geq$ 125%) since this would represent a prescreen. Existing data on crude plants indicates that daily treatment would produce a greater yield than q4day treatment. These should also be screened in cell culture (KB). From September 1971 through August 1972, 78 crude plant extracts showed confirmed activity (T/C% $\geq$ 125) in P388 and 74 were active in KB. Of the 74 active in KB, 17 were active against P388. The remaining (57 in this year's experience) would be prime candidates for screening against other experimental signal tumors. In CY 1972, 10,434 plants were submitted for screening. One

hundred fifty-two materials showed confirmed activity in P388 and/or KB. Such plants are scheduled for recollection. It is estimated that less than half of the crude extracts of recollected samples show activity, suggesting that greater selectivity be practiced in determining which plants should be recollected.

Step 2. Test P388 actives and KB actives from Step 1 (crude re-extracts of the original collection) against IP-P388 and IP-L1210 using a more definitive protocol (two treatment schedules and 6 mice/gp.). Demand confirmed activity against P388 or L1210 to go on to Step 3. Permit limited fractionation of KB actives that are not active against P388 or L1210 and test the most active fraction against P388, L1210, B16, LL, and other signal tumors that might emerge. While this may seem like a considerable amount of testing, it really wouldn't be costly because only about 200 materials a year would be expected to emerge from the Step 1 pre-screen. No plant recollection and almost no fractionation would have been involved. Use of the Steps 1 and 2 procedures as outlined may be expected to reduce the number of "confirmed actives" to 50-60 per year. This would reduce recollection and fractionation costs to about 43% of current costs.

Step 3. Recollect, re-extract and confirm activity of those emerging as active from Step 2.

Step 4. Permit fractionation of in vivo actives from Step 3 using KB primarily if appropriate and/or one in vivo system. Control the fractionation for each from within DR&D. Establish a scale that limits the number of fractions permissable as a function of degree of activity and progressive increase in activity. Permit fractionation leading to isolation as long as current criteria for passing DN-2A are approached.

II. Screening and Fractionation of Fermentation Products

Less than 10% of the materials submitted for screening in CY 1972 were crude fermentation products. Significantly, of the approximate $970,000 total effort in in vitro and in vivo bioassay (L-1XX), about $260,000 is funded within the in-house effort at Bristol Labs (Contract 35) and $97,000 at Upjohn (Contract 1023). Most of this involves fermentations. The total screening (not bioassay) in vitro and in vivo effort (L-2AX) at Bristol is funded at about $87,000, and at Upjohn - $41,589. A small amount of fractionation bioassay is being done elsewhere for Upjohn and some of Upjohn's screening is on synthetics. It is noteworthy that 58% of all L-1XX in vitro bioassay and 30% of all L-1XX in vivo bioassay is expended in these two in-house projects. DR&D is reviewing the entire fermentation screening program in detail. Since DEB does not have primary responsibility for monitoring these in-house projects, it is difficult to make specific recommendations at this time. In general, it would seem appropriate to apply the same rigid mechanism as proposed for the plant program. However, crude fermentation beers are subjected to some kind of prescreening. It would appear that considering the modest input of these materials into program and the apparent high level of fractionation bioassay, much of which may involve studies of variations in temperature and

medium, DR&D should continue its current policy of increasing intramural staff control of this area.

III. Primary Screening of Synthetic Materials (L-2AX)

A. Screening of small samples - the L1210 "Mini-Screen". The work done on this project is properly classified as exploratory (E-2AX), and the results have implication to adjustments in routine screening protocols. Two laboratories, Microbiological Associates, Inc. (MAI-3728) and A. D. Little (ADL-3727) were selected to test the mini-screen as models. The overall design is summarized in the current Individual Project Report-3576. Basic changes from the current L1210 screen were (a) use of 3 mice per test group rather than 6, (b) sc tumor implantation rather than ip, (c) sc treatment rather than ip, (d) use of one treatment schedule, days 3 and 7, rather than two; qd, Days 1-9 and q4d, Days 1,5,9, (e) use of 6-10 dosage levels rather than 3, (f) use of tumor size in addition to survival as a second parameter of response. Seven drugs of established anti-L1210 activity and three known inactives were submitted to both labs. Each drug we submitted in 7 different amounts ranging from 5.0 to 150 mg. Thus, the basic test for each lab involved the screening of 70 coded materials. The results from both labs were analyzed by Dr. I. Miller (ADL-3727). There was no essential difference between results from each lab. The results indicated that (a) the mini-screen was feasible, (b) it does offer a considerable saving in drug utilization, (c) known active compounds were effectively distinguished on the basis of survival results when the amount submitted was 20 mg. or more, (d) use of tumor size as a parameter led to an increase in false positives, (e) use of survival alone did not reduce the number of true positives. With respect to relative cost efficiency, MAI reported the mini-screen as conducted required 0.43 man hours per test group while our current routine L1210 screen requires 0.56 man hours per test group in that lab. It should be emphasized that the mini-screen was designed to increase efficiency of drug utilization and not as a means of decreasing screening costs. However, proper modification of the mini-screen as part of an overall modification of our in vivo primary screening program could conceptually permit the screening of more materials and permit us to concentrate more on developing the most active ones (see this report, Part IIIC).

B. Current Screening Program - At present, synthetics are screened against L1210 using 1 or 2 treatment schedules depending on the amount of material available. Those available in amounts of less than 65 mg. are not screened in vivo. Clearly, the mini-screen approach would permit in vivo screening of most of the latter. Currently, the cost of L-2AX (L1210, P388) primary screening in the labs concerned is approximately $3,600,000 per year; roughly half being devoted to synthetics (L1210) and half to crude natural products (P388). During CY 1972, 375 materials showed confirmed activity with the minimal activity required for review by the Drug Evaluation Committee (DEC). This figure includes synthetics and purified materials from natural products since the latter are handled identically to synthetics when the pure drug is isolated. Thirteen materials were recommended to the Assoc. Sci. Dir., DR&D as potential

candidates for DN-2A consideration. Nine agents passed DN-2A (see Screening Section Report, Tables I and II). Of the nine, one passed on the basis of clinical activity, one on the basis of reverse transcriptase activity, 2 were superior analogs of agents that had previously passed DN-2A, and 5 represented novel agents (2 synthetics active against L1210, one fermentation derivative active against L1210, and two derivatives of one plant active against P388). The attrition between the observation of confirmed minimal activity and recommendation for DN-2A consideration is largely due to failure to meet the more stringent criteria for DN-2A passage. However, a significant number of those meeting DN-2A criteria are analogs of clinically active drugs without demonstrated superior activity. Steps should be taken to provide for (a) increasing the input of new structures into the screen, (b) increasing the input and quantitative evaluation of congeners of very active clinical drugs, (c) decreasing or eliminating the input of structural types in which there is no clinical interest, (d) eliminating inactive agents more efficiently, and (e) concentrating more comprehensive testing of active novel agents or structural types of clinical interest prior to DN-2A review.

C. Recommendations

Stage I. Pre-screen all synthetics against IP-L1210 using a modification of the mini-screen protocol. Reduce the number of mice per test group to 3. The parameter for activity should be increase in mean lifetime over controls. However, activity should be confirmed before proceeding. One moderately long survivor might indicate false activity. If we use the median survival time, one moderately long survivor wouldn't count in summarizing the results and a true active might be lost. The problem of the yield of false actives (agents that would not retain activity in more definitive testing) could be significant if we need to expend funds to synthesize a great number of these. It would be better to use IP treatment of IP tumor rather than SC treatment of SC tumor at this stage since it really represents a pre-screen to quickly eliminate most of the inactives. Two treatments spaced 4 days apart (e.g., days 2 and 6) at 4 dosage levels would be adequate. Based on the following estimates such a screen would eliminate an inactive compound for about one-half the current cost of animals and biological testing labor. It is a modification of the "Econo-Screen" as proposed by Dr. A. Goldin. Of course, it should be realized that while the use of this approach would permit the screening of significantly more synthetic compounds without increasing costs within the screening lab or the cost of animals, it would not be realistic to assume that the input could be doubled using our current mechanisms for identifying, classifying, assigning NSC numbers, and shipping compounds; and computer processing of the data. Any increased input would increase the costs of these activities proportionately because for each compound, these costs are independent of screening and animal costs. Perhaps some modification of these operations should also be considered.

In CY 1971, about 15,000 new materials (synthetics plus natural products) were submitted to program and 195 displayed confirmed minimal activity. In CY 72, about 30,000 materials were submitted and 375 showed confirmed minimal activity. It is appropriate to consider the total input inasmuch

as confirmed active materials include purified natural products. It should also be borne in mind that the actives were not necessarily among those submitted in any one year. Nevertheless, the yield of confirmed actives appears to be about 1.3%. Suppose initial use of the Stage I protocol increased this rate to 2.0% and the input remained at a level of 30,000 per year. If 98% of the materials (29,400) are eliminated in Stage I, we could concentrate our efforts on the 600 remaining materials as described below (Stage II). Alternatively, if the input were increased to 60,000, we would have about 1200 remaining for more intensive study.

Stage II. (Synthetics emerging as active from Stage I and purified natural products). Our hypothetical 600 materials will still need to be screened definitively against L1210 or P388. It would be inappropriate to consider formulation development and toxicology on the basis of Stage I results or bioassay data. Since the number of materials would be small, they could be tested very adequately using 10 mice per treatment group, 4 or 5 dosage levels, and 2 or 3 treatment schedules in the following systems:

(1) IP-L1210 and/or P388 on 3 treatment schedules
(2) SC-L1210 and/or P388
(3) IP-B16
(4) SC-B16
(5) SC-LL
(6) Route dependency in any sensitive system
(7) Other appropriate host-tumor systems that might be developed
(8) Normal mouse toxicity

Most of our current S-2AX testing and about 20% of our current L-2B2 (Detailed Drug Evaluation) testing is included in the proposed Stage II testing. However, it seems that the results of the Stage II tests would permit a more informed review at DN-2A and consequently better selections of agents for development. It is also recommended that we establish a mechanism for the regular surveillance of the literature and reports from other screening programs and enter agents with reported activity (any system) directly into Stage II screening.

IV. Screening of Contractor Synthesized Analogs

During the last year, a mechanism was established to have one laboratory (MAI-3728) test these materials in comparison with the parent compound using protocols designed for each specific structural type. (See current Individual Project Report - Serial No. 3583) The results are reported to our data processing contractor but also to the compound supplier directly. The project is working well to the satisfaction of our suppliers. Now that this mechanism for specifically directed analog testing has been established, a number of actions should be taken:

A. Program should continually review the analog synthesis program to maintain emphasis on current clinical interests.

B. A committee should be formed to review the protocols and modify them to permit maximum ability to select new agents from among analogs.

Biochemical, pharmacological and biological tests as well as the physical-chemical characteristics of the analog should be considered. These tests should be specific for each of the compound types. Priorities among assays should be established based on the characteristics of the parent compound.

C. A mechanism should be provided to identify any submitted analog of an active agent of interest and subject contract generated and voluntary ("random") submissions to identical comparative studies.

V. New Screening Assays

Examination of Tables 1 and 3 shows a considerable effort in the development of new assays for screening. A large portion of this work involved the evaluation of IP and SC-B16 as model screens, the 1st generation AKR system at IITRI (1021) and the mini-screen. Table 5 shows that the B16 system would identify a number of clinically active agents that do not meet DN-2A criteria against L1210 (Bleomycin, VCR, VLB, Act. D). B16 is also considerably more sensitive than L1210 to Adriamycin and Daunomycin and less sensitive to S-phase inhibitors. Bleomycin, VCR, VLB, Act. D are effective against P388. Thus, the overall screening plan of using P388 and L1210 as initial screens for natural products and synthetics, respectively; to be followed by broader spectrum screening as outlined earlier seems reasonable. The 1st generation AKR assay results correlate well with activity in the spontaneous AKR system. However, inasmuch as the spontaneous AKR seems to be most appropriate for concomitant and sequential combinations and is not being considered for identification of new active agents, except for prophylaxis, the objective of the 1st generation assay (a new agent pre-screen for candidates for spontaneous AKR testing) is no longer pertinent. The system can be phased out or used as a Stage II screen (see Primary Screening of Synthetic Materials, recommendations, Stage II). We should put some effort into developing assays for screening against particular tumor types in order to determine if positive correlations with clinical responses might exist. A good source of such tumors might be the carcinogenesis testing program where at least one, a transplantable colon tumor, has arisen.

VI. Detailed Drug Evaluation

Studies leading to the development of agents emerging as active from the primary screen may be further segregated into one of three broad categories (Tables 1-3); those that provide information directly concerned with the progress of a drug through the linear array (L-2B2); those which provide useful ancillary information about the characteristics of a drug (S-2B2); and those designed to explore new assay systems for detailed drug evaluation (E-2B2). L-2B2 studies include route and schedule dependency, normal mouse toxicity, comparisons of active congeners, biological quality control, combination chemotherapy in the screening assay that uncovered the drug, activity in other experimental established in vivo assays, and activity against advanced tumors. S-2B2 studies include activity in ancillary in vivo systems, effects on host defense mechanisms, CXT studies in vitro, cell kill kinetics, and biochemical mechanisms of action, etc.

E-2B2 studies involve the development of animal assays as models for human tumors. For purposes of the analysis presented in Tables 1-3, all in vivo screening with selected agents (SAC) compounds was included in L-2B2. While such a classification system is somewhat arbitrary, it was necessary in order to generate a firm estimate of relative effort among the various DCT-array activities. Hopefully, within the next year we will be able, with the cooperation of the laboratories concerned, to generate a more detailed breakdown of effort (e.g., schedule dependency vs. combination chemotherapy studies).

Examination of Tables 2 and 3 shows that the more routinized L-2B2 studies are all related to in vivo testing and are conducted in nearly all of the contract laboratories conducting such tests. However, L-2B2 testing constitutes a substantial part of the effort at Southern Research (2098), ADL (3727), and the DEB-monitored effort at MAI (3728). The less routinized S-2B2 in vivo testing is concentrated largely at these same three laboratories. S-2B2 in vitro studies relate primarily to the CXT characteristics and cell cycle stage specificity of active drugs. E-2B2 work in vivo and in vitro is concentrated largely in laboratories conducting the bulk of the S-2B2 studies with the exception of the project at Catholic Medical Center (3703) which is aimed at development of a mammary carcinoma assay for combined modalities of treatment; chemotherapy, surgery and immunotherapy.

It is significant that detailed drug evaluation-related projects (L,S, and E-2B2) at Southern Research (2098 and 29), MAI (3728) and ADL (3727) are funded at approximately $2,500,000 representing 74% of the total 2B2 effort and 27% of the total DEB-monitored contract effort. Overall, 5 contracts at these three laboratories (Southern Research 2098, 2189, 29; MAI-3728; ADL-3727) represent approximately 49% of the total funding of DEB-monitored contract projects. Major findings within the Drug Evaluation Division and Immunology Division, MAI (3728) are described in the current Individual Project Reports, Nos. NCI-3583 and 3577, respectively. Accomplishments under the Southern Research Institute and ADL contracts are reported in the contractors annual and interim progress reports.

Studies related to detailed drug evaluation are diverse in nature, but there has been considerable emphasis on combination therapy. Examples of therapeutically advantageous drug combinations against some animal tumors have included:

Adriamycin (NSC-123127) + DTIC (NSC-45388)	L1210 B16 C3H, MaCa
Cytoxan (NSC-26271) + MeCCNU (NSC-95441)	B16 Spont. AKR C3H MaCa
VCR (NSC-67574) + Prednisone (NSC-10023E)	Spont. AKR LL

Ara-C (NSC-63878) + 6-Thioguanine (NSC-752)	L1210
Ara-C + 6-Mercaptopurine (NSC-755)	L1210
Ara-C + CCNU (NSC-79037)	L1210
Ara-C + Procarbazine (NSC-77213)	L1210
Anhydro-Ara-C (NSC-145668) + Procarbazine	L1210
Daunomycin (NSC-82151) + ICRF-159 (NSC-129943)	L1210
Adriamycin + ICRF-159	L1210
Daunomycin followed by Ara-C	L1210
Adriamycin + Methotrexate (NSC-740)	L1210
CCNU + BIC (NSC-82196)	L1210
Adriamycin + Me-CCNU (NSC-95441)	B16

The group at Southern Research reported a number of 2, 3, and 4 drug combinations to show a therapeutic advantage against spontaneous AKR lymphoma.

The interim DEB contractor's workshop was held at the Southern Research Institute, Birmingham, Alabama on March 19-20, 1973. The two-day meeting was devoted entirely to the question of combination therapy with emphasis on (a) rationales for selection of combination modalities, (b) experimental design, (c) data analysis and evaluation, and (d) data retrieval and processing. It is anticipated that the proceedings will be published in Cancer Chemotherapy Reports (Part II, July 1973 issue).

The group at Southern Res. (29) observed that 3-Deazauridine (NSC-126849) inhibition of the growth of Ca-755 cells in culture was partially blocked by deoxycytidine (NSC-83251) suggesting that 3-Deazauridine might inhibit formation of deoxycytidine ribonucleotides. Subsequently, the group working under Southern Res. contract 2098 found that an Ara-C resistant subline of L1210 was much more sensitive to 3-Deazauridine therapy than was the parent sensitive line. The combination of Ara-C + 3-Deazauridine is currently under study.

In general there has been increased emphasis on the application of information relative to biochemical mechanisms of drug action and studies of resistance and cross-resistance to the prediction of potentially advantageous drug combinations. In addition, we have been interested in studying the effects of known active drugs at some potentially important biochemical sites that may not have been adequately explored. To these ends we have within the last two years initiated a number of modest projects. Since the test systems available and suggested for such laboratory studies are many and diverse, DEB felt the need to establish an ad hoc committee to (a) act as a source evaluation group to review unsolicited contract proposals in the areas of biochemistry and molecular biology, (b) provide advice and guidance in the conduct of related contracts, (c) plan collaborative projects with other DR&D and NCI contract activities, (d) plan and conduct workshops on related aspects of DR&D program objectives, and (e) initiate comprehensive files on chemical and biochemical properties of drugs of interest. The Biochemistry Committee was organized under the direction of the Assoc. Sci. Dir., DR&D, and the Chief, DEB. It has functioned under the Chairmanship of Dr. Vincent H. Bono, Jr., with the collaboration of Dr. Florence R. White,

Head, Biochemistry Section. The Chairman's summary report follows:

VII. Summary Report of the Biochemistry Committee for DR&D (Also see current report, Biochemistry Section, DEB).

A. Unsolicited contract proposals

The Committee reviewed unsolicited contract proposals in the following areas: effects of combinations of drugs on the growth of cells in cell culture, effects of drugs on cell culture strains of human colon carcinoma, effects of drugs on the synthesis of major macromolecular species in cell culture and detailed studies on associated processes as indicated, and effects of drugs on enzyme activities in vitro. These proposals are on record as potential resources for application to DR&D program objectives.

B. Ad Hoc Biochemistry Contract Review Committee

Since the decision to discontinue the standing contract review committees, the Biochemistry Committee has taken the responsibility of forming ad hoc contract review committees to review Biochemistry and Molecular Biology contracts. During the past year it has organized two such review committees.

C. Advice and Guidance in the Conduct of Biochemistry and Molecular Biology Contracts

NIH-69-2115 (Delaware U.). Suggested the following two goals be set for the current contract year: (1) To provide a method for purifying Aerobacter glutaminase that is at least 90% enzyme protein and utilize methodology applicable to large scale preparations and (2) to provide 4,000 to 10,000 I.U. of purified enzyme activity or provide clear evidence that it can be provided without further significant modifications in methodology.

NIH-71-2189 (Southern Res. Inst.). Suggested that the methodology that has now been developed to determine if a drug selectively inhibits phospholipid biosynthesis as compared to DNA biosynthesis in L1210 cells in cell culture, be used to evaluate all drugs in clinical trial and those which have passed DN-2A.

NIH-71-2188 (Pennsylvania U.). (A) Suggested that the observation that daunomycin uncouples oxidative phosphorylation in isolated mitochondria from pigeon heart at significantly lower concentrations than in mitochondria from rat liver, be confirmed in heart and liver mitochondria from the same species (rat) and that it be extended to the effects of adriamycin and ellipticine. There is a possibility that these observations may relate to the cardiac toxicity of daunomycin and adriamycin and may be applicable to the evaluation of their analogs. (B) Suggested that a system be used to determine the effect of drug administration on aerobic respiration and the Crabtree Effect in L1210 cells in vivo and that the following drugs be evaluated: Daunomycin (82151), Adriamycin (123127),

Ellipticine (71795), CCNU (79037), MeCCNU (95441), Tritylcysteine (83265), ICRF-159 (129943), cis-platinum diammine dichloride (119875), Bleomycin (125066), Neocarzinostatin (157365), and Tetrandrine (77037). As a form of control the following drugs will also be evaluated: 5-fluorouracil (19893), cytosine arabinoside (63878), 5-hydroxy-picolinaldehyde thiosemicarbazone (107392), 6-MP (755), and Actinomycin D (3053).

NIH-71-2339 (Yale U.). Suggested that the following program drugs be studied for (a) binding strength with native DNA and (b) effects on T_m of native DNA and complexes of prA-prU, prA-pdT and pdA-pdT: Coralyne (96349), "Cain's Acridine" (141549), Nitidine (146397), Ellipticine (71795), Hycanthone (142982), Daunomycin (82151), Adriamycin (123127) and Chromomycin A3 (58514).

PH-66-29 (Southern Res. Inst.). The Biochemistry Committee met with the principal investigators in October 1972 to discuss the research objectives of the group, prior to their submitting a proposal for renewal of their contract for the year starting February 28, 1973. The scope of their proposed work is extensive but falls into several major categories (a) initial evaluation of agents for effects on nucleic acid and protein synthesis (for agents that have passed DN-2A), (b) detailed studies on the biochemistry of agents in clinical trials or being developed for clinical trials, (c) the use of HEp-2 purine enzyme deletion mutants to evaluate the activation pathway for active purine analogs and to allow identification of those analogs having an unusual activation pathway, and (d) the development of a similar series of HEp-2 pyrimidine enzyme deletion mutants to allow a similar evaluation of pyrimidine analogs.

NIH-CR-(71)-56 (AEC). Suggested that the following drugs be evaluated for selective effects on the progress of CHO cells through the proliferative cell cycle: Cis-Pt-II (119875), Chromomycin A3 (58514), Thalicarpine (68075), VP-16 (141540), Adenosine arabinoside (404241), Tetrandrine (77037), Hycanthone (142982), CCNU (79037), BCNU (409962) and MeCCNU (95441).

NIH-72-2042 (Scripps Clinic). The objective of this research is to synthesize analogs of vitamin B12 and to determine if they have (a) effects on B12 dependent enzymes systems in vitro, (b) effects on L1210 cells in cell culture and (c) therapeutic activity in an animal tumor system. So far a reasonably large series of analogs have been prepared and chemically characterized. Some of them have inhibitory effects on ribonucleotide reductase from L. leichmannii and some of them interfere with the uptake by L1210 cells of vitamin B12 in cell culture. This latter effect depends on the addition of transcobalamin II, which is a transport protein for vitamin B12. To date, the only cytotoxicity demonstrated has been with the cytosine arabinoside analog of vitamin B12. It is believed that Ara-C B12 undergoes photolytic decomposition to the 5' aldehyde of free cytosine arabinoside and that this species may be responsible for the cytoxicity seen.

(D) Collaborative Projects with DR&D and Non-DR&D Contract Activities

(1) Working Group for Molecular Controls. Plans are underway to screen all program drugs past Decision Network 2A for inhibition of several DNA and RNA dependent DNA polymerases under a new contract for Screening of Drugs for Antitumor and Antiviral Activity.

(2) Ash Stevens Inc. (Dr. M. Sporn - Project Officer) - Plans are underway to obtain small quantities of nucleotide and oligonucleotide phosphodiesters with covalent bond forming abilities so that they may be evaluated for cytotoxicity in cell culture. Significant cytotoxicity in cell culture would be evidence of their ability to enter cells and an indication that we should seriously consider supporting synthesis of this type of agent.

(3) NIH-CR-(71)-56 (A.E.C. Biomedical Group) - In collaboration with Dr. Robert Tobey, an intramural group (Dr. Vincent Bono) is evaluating the application of flow monofluorometric determination of DNA content distributions to the effects of drugs on log phase L1210 cells in cell culture. The successful application of this methodology might allow (a) biological characterization of series of active structural analogs and (b) further biological characterization of agents exhibiting significant cytotoxicity in cell culture. A second plan being considered is to explore the possibility of applying similar methodology to characterizing the proliferative kinetics of tumors used in primary and secondary screening. The AEC Biomedical Group is interested in such a proposal.

(E) Plan and Conduct of Workshops on Biochemical Aspects of DR&D Program Objectives

A workshop on Direct Interaction, Metabolic and Biological Effects of Nitrosoureas was held 13 September 1972. A summary of the major facts presented will follow. Nitrosoureas have both alkylating and carbamylating activity. There is no clear indication that either of these two activities is primarily responsible for the therapeutic activity of these compounds. The extent of carbamylation quantitatively far exceed the extent of alkylation. Carbamylation is principally of proteins and alkylation is of nucleic acids. Selectivity of carbamylation has been demonstrated: BCNU reacts extensively and rapidly with the serum albumin in serum protein. Nitrosoureas interfere with the normal processing of 45s precursor of r-RNA and of heterogeneous nuclear RNA. Selective inhibition of methyl group utilization from histidine for de novo purine biosynthesis has been demonstrated. Cells in culture are most sensitive to the effects of BCNU in late G1 and early S phase but are susceptible in all phases of the cell cycle. Cells that survive BCNU in tumor bearing animals have increased durations of S phase and T_c. In summary, the nitrosoureas are chemically reactive compounds that have selective interaction and metabolic effects in cells. As yet, the biochemical effects responsible for their biological activity have not been found.

(F) Establishment of Files on the Chemical and Biochemical Properties of Program Drugs

During the past year, a modest beginning was made to collect relevant information on program drugs. The Drug Development Branch has provided information on the solubility and stability characteristic of program drugs. The Program Analysis Branch has provided bibliographies on program drugs. It is anticipated that a service file on the chemical, biochemical and molecular biological properties of program drugs may be established.

Committee Membership

Dr. Vincent H. Bono, Jr., Chairman
Dr. Theodore Breitman
Dr. David Cooney
Dr. Abraham Goldin
Dr. Herbert E. Kann
Dr. Kurt Kohn
Dr. J. A. R. Mead
Dr. Marco Rabinovitz
Dr. Saul Schepartz
Dr. John Venditti
Dr. Florence White, Executive Secretary

VIII. DEB Intramural Projects (See current Individual Project Reports, Nos. NCI-3576, 3583, 3577, and 3578).

The objectives of these projects are summarized in Table 4. Projects 3576 and 3577 represent collaborative efforts between DR&D staff and contract 3728 (MAI). Progress of Project 3576 has been discussed in other sections of this report. Progress of project 3577 is discussed in the report of the Assoc. Sci. Dir. for DR&D by the Assoc. Chief for Laboratory Research, Dr. A. Goldin, who acts as principal advisor for this project.

Project 3583 (Dr. Lippman) is aimed at modifying patterns of cellular division and immunogenicity of tumor cells by interaction of their surfaces with micro-environmental macromolecules. Part of this overall objective involved development of in vivo assays permitting a quantitative assessment of tumor cell immunogenicity, host immunological responses, and the effects of drugs thereon. It was found, for example, that injection of live mouse strain A/He specific TA3K1 tumor cells into BDF1 mice rendered them immune to subsequent challenge with non-strain specific TA3Ha cells at inoculum levels that kill 100% of unimmunized BDF1 mice. Depending on the TA3K1 inoculum level, one of three responses were achieved: immunity, prolongation of survival, or reduction of survival. The results indicated that effective immunogenicity rather than immune resistance on the part of TA3Ha cells was the major factor in permissive growth of the latter in histocompatible animals. Such results bear on problems in applying immunotherapy; inasmuch as immunotherapy may be a function of the tumor burden and vary in effectiveness depending on prior

drug treatment, radiation or surgery designed to reduce the tumor cell burden, and on the effect of these treatments on host defense mechanisms. The TA3 system appears to be suitable for measuring in vivo immunosuppressive or immunoaugmentative action of drugs. In collaboration with Drs. R. Folk and A. Barker (BMI, not contract supported), the TA3 system was used to rank the immunosuppressive action of 4 drugs of interest. It was found that, with respect to immunosuppressive activity, BCNU > Cytoxan > MeCCNU > CCNU. While relative activity was a function of the tumor challenge, it was clear that BCNU was the most suppressive of these drugs.

With respect to studies with surface binding agents, it was found that Macromomycin was bound to malignant cell surfaces and blocked division of L1210 and TA3Ha cells in culture. Effective inhibitory concentrations did not agglutinate the cells but increased the population of cells of the size equivalent to metaphase or doublet cells (See current Individual Project Report, Serial No. NCI-3583). Project 3578 (B. J. Abbott) is directed at the conduct of basic studies related to in vivo antitumor screening emphasizing the rapid solution of problems arising during formulation development and toxicological evaluation of active drugs; and on the establishment of protocols and parameters for screening. With respect to the latter objective, this laboratory project addressed itself to basic questions of procedures for screening large numbers of agents and applied its findings to its instructions to contract screening laboratories. For example, the toxicity to mice of commonly used vehicles for drug delivery is studied especially with respect to some of the intensive treatment schedules used for the detailed evaluation of active drugs. The establishment of suitable protocols for the preparation of unknown drugs for injection is important to a large-scale screening program because many vehicles that can be used safely in larger animals do elicit toxic responses in mice. In collaboration with contract project 1026 (IITRI), this intramural project continued to evaluate drugs against first generation AKR lymphoma. The overall findings of this collaborative study were discussed earlier in this report. Other tasks conducted within this intramural project are described in the current Individual Project Report, Serial No. NCI-3578.

IX. Future Course of DEB Program

The DEB program will continue to devote its efforts to the screening of new agents for potential clinical usefulness. New assay development will emphasize the degree to which screening procedures will uncover drugs of value against human solid tumors. At the same time, we hope to modify current predictive systems to further increase the efficiency of their utilization. Consequently, we expect to be able to accommodate the expected increased input of synthetics to the primary screen without increasing the cost of the testing program. It would appear that we have defined a sufficient number of in vivo experimental assays to predict nearly all the drugs now known to be effective against human solid tumors as well as leukemias and lymphomas. While no one system alone seems capable of doing this, the efficient use of available systems in appropriate sequence would appear to meet our desired

screening goal. It remains then for us to develop more efficient systems as initial or pre-screens in order to economically segregate, from among many thousands of materials, those that are most likely to meet our criteria for development to clinical trial. In the area of new assay development, massive studies of correlations among host-tumor assays will be deemphasized in favor of basic developmental studies of other kinds of systems (e.g., organ cultures, specific cytotoxicity tests, etc.) that may be both advantageous and economical. Thus, the primary screening program will be aimed at the rapid elimination of materials with no apparent potential for activity and more comprehensive study of agents with clinical potential. In this way we hope to utilize resources for more complete study in secondary screens of the 1-2% of the agents of greatest potential. Agents that pass more definitive screens (perhaps 0.1% of the total screened) will continue to be recommended as candidates for clinical trial and be studied in depth with respect to route and schedule dependency, activity against advanced tumors, activity in combined modalities of therapy, mechanisms of action, and activity in specific organ directed secondary screens.

Because of the profound success of drug combinations in the clinic, preclinical investigative work in this area will continue to be emphasized. However, it will be necessary to apply rigid criteria in selecting drugs for combination studies because of the relatively high cost of such studies and the great number of possible combinations that may be envisioned. At the same time, we hope to increase our effort to achieve cures in experimental tumor systems by the use of drugs, surgery, and/or immunotherapy.

Table 1
Distribution of DEB Monitored Contract Projects by DCT-Array[1/]

Array	Linear L	Supplementary S	Exploratory E	Total
		(Amount, $)		
1XX	973,598	0	102,723	1,076,321
2AX	3,666,318	144,113	1,042,459	4,852,892
2B2	1,441,433	1,186,075	838,022	3,465,530

	Project	(Amount, $)
L-1XX	Bioassay *in vitro*	283,090
L-1XX	Bioassay *in vivo*	660,671
L-1XX	Bioassay - Enzyme Production	20,495
E-1XX	Bioassay - *in vitro* - New Assay Development	12,478
E-1XX	Bioassay - *in vivo* - New Assay Development	11,745
E-1XX	Biochemical Tests Aimed at New Agent Synthesis	78,500
L-2AX	Screening - *in vitro*	333,623
L-2AX	Screening *in vivo* (Current Systems)	3,309,329
S-2AX	Screening - *in vivo* (Non-current, Established Systems)	128,033
S-2AX	Screening - Biochemical	16,080
E-2AX	Screening - *in vitro* - New Assay Development	48,521
E-2AX	Screening -*in vivo* - New Assay Development	837,369
E-2AX	Tumor Cell Kinetics - *in vivo*	123,613
E-2AX	Screening - Biochemical - New Assay Development	32,956
L-2B2	Detailed Drug Evaluation - *in vivo* - Established Systems	1,427,420

Table 1 (Cont'd.)
Distribution of DEB Monitored Contract Projects by DCT-Array[1/]

	Project	(Amount, $)
S-2B2	Detailed Drug Evaluation - *in vitro*	207,977
S-2B2	Detailed Drug Evaluation - Biochemical Mechanisms	325,536
S-2B2	Detailed Drug Evaluation - *in vivo* - Special Assays, Immunology	652,562
E-2B2	Detailed Drug Evaluation - *in vitro* - Assay Development	25,472
E-2B2	Development - Animal Models of Human Tumors	812,550
L-1XX	Tumor Bank	9,342
L-2AX	Tumor Bank	23,366
L-2B2	Tumor Bank	14,013
TOTAL - DEB Monitored Projects		9,394,741

1/ Funding based on amount negotiated for FY '73, if any, or on amount obligated in FY '72 as of the January 31, 1973 DCT Monthly Report of Contract Commitments.

Table 2
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project (Code)	(%)	($)
ATOMIC ENERGY COMMISSION 71-56		100	21,000
Detailed drug evaluation - in vitro	S-2B2	50.0	10,500
Related assay development	E-2B2	50.0	10,500
BATTELLE MEMORIAL INSTITUTE 71-2182		100	471,640
Bioassay - in vivo	L-1XX	0.1	472
Screening - in vivo	L-2AX	77.9	367,407
Related new assay development	E-2AX	4.0	18,866
Detailed drug evaluation - in vivo	L-2B2	18.0	84,895
BRISTOL - MYERS CO. 69-35*		54.0	387,104 716,859
Bioassay - in vitro	L-1XX	15.4	110,396
Bioassay - in vivo	L-1XX	21.8	156,275
Screening - in vitro	L-2AX	4.0	28,674
Screening - in vivo	L-2AX	8.2	58,782
Related new assay development	E-2AX	3.6	25,807
Detailed drug evaluation - in vivo	L-2B2	1.0	7,169
CATHOLIC MEDICAL CENTER 72-3703		100	182,646
Develop. - Animal models of human tumors	E-2B2	100	182,646
DELAWARE UNIVERSITY 69-2115		100	20,495
Bioassay - Enzyme Production	L-1XX	100	20,495
HAZLETON LABORATORIES 72-3704		100	698,100
Bioassay - in vivo	L-1XX	14.0	97,734
Screening - in vivo	L-2AX	56.0	390,936
Screening - in vivo	S-2AX	2.0	13,962
Related new assay development	E-2AX	2.0	13,962
Detailed Drug Evaluation - in vivo	L-2B2	21.0	146,601
Detailed Drug Evaluation - in vivo	S-2B2	5.0	34,905

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project (Code)	(%)	($)
IIT RESEARCH INSTITUTE 68-1021		84.0	1,204,799 1,434,284
Bioassay - in vivo	L-1XX	9.5	136,257
Screening - in vivo	L-2AX	47.0	674,113
Related new assay development	E-2AX	13.5	193,628
Detailed drug evaluation - in vivo	L-2B2	14.0	200,800
INSTITUTE MARIO NEGRI 73-3720		80.0	99,354 124,192
Screening - in vivo	L-2AX	80.0	99,354
JAPANESE FDN. CANCER RESEARCH 72-2054*		25.0	14,125 56,500
Screening - in vivo	L-2AX	25.0	14,125
LITTLE, ARTHUR D. 73-3727*		59.0	1,377,971 2,335,544
Bioassay - in vitro	L-1XX	1.5	35,033
Related new assay development	E-1XX	0.5	11,678
Bioassay - in vivo	L-1XX	0.3	7,007
Screening - in vitro	L-2AX	1.5	35,033
Screening - in vivo	L-2AX	18.0	420,398
Screening - in vivo	S-2AX	2.0	46,711
Related new assay development	E-2AX	8.6	200,857
Detailed drug evaluation - in vitro	S-2B2	2.4	56,053
Related new assay development	E-2B2	0.2	4,671
Detailed drug evaluation - in vivo	L-2B2	13.0	303,621
Detailed drug evaluation - in vivo	S-2B2	4.0	93,422
Develop. - Animal models of human tumors	E-2B2	5.0	116,777
Tumor bank	L-1XX	0.4	9,342
Tumor bank	L-2AX	1.0	23,355
Tumor bank	L-2B2	0.6	14,013
MASON RESEARCH INSTITUTE 71-2259		92.9	520,119 559,870
Bioassay - in vivo	L-1XX	0.2	1,120

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project (Code)	(%)	($)
MASON RESEARCH INST. 71-2259 (Cont'd.)			
Screening - in vivo	L-2AX	68.5	383,511
Related new assay development	E-2AX	2.2	12,317
Detailed drug evaluation - in vivo	L-2B2	22.0	123,171
MIAMI, UNIV.OF 70-2225		100	129,145
Bioassay - in vitro	L-1XX	0.7	904
Screening - in vitro	L-2AX	99.3	128,241
MICHIGAN DEPARTMENT HEALTH 73-3702		100	45,115
Bioassay - in vitro	L-1XX	100	45,115
MICROBIOLOGICAL ASSOCIATES 73-3728*,+		41.0*	824,925* 2,012,012
Screening - in vivo	L-2AX	13.0	261,562
Screening - in vivo	S-2AX	1.3	26,156
Related new assay development	E-2AX	8.1	162,973
Detailed drug evaluation - in vivo	L-2B2	8.0	160,961
Detailed drug evaluation - in vivo	S-2B2	6.6	132,793
Develop. - Animal models of human tumors	E-2B2	4.0	80,480
PENNSYLVANIA, UNIV. OF 71-2188		100	11,072
Screening-Biochemical-new assay develop.	E-2AX	50.0	5,536
Detailed Drug Evaluation - Biochemical Mech.	S-2B2	50.0	5,536

+22% - Drug Evaluation Division - Monitored by DEB
10% - Immunology Division - Monitored by DEB and Office of Assoc. Sci. Dir. DR&D
9% - Chemotherapy Research Division - Monitored by Office of Assoc. Sci. Dir. DR&D

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project (Code)	(%)	($)
RESEARCH TRIANGLE 69-2019*		18.0	25,194 139,968
Bioassay - in vitro	L-1XX	15.0	20,995
Screening - in vitro	L-2AX	3.0	4,199
SCRIPPS CLINIC 72-2042		50.0	23,500 47,000
Biochemical tests aimed at new agent synthesis	L-1XX	50.0	23,500
SOUTHERN RESEARCH INSTITUTE 66-29		100	320,000
Detailed drug evaluation - Biochemical Mech.	S-2B2	100	320,000
SOUTHERN RESEARCH INSTITUTE 71-2098		100	2,060,223
Screening - in vitro	L-2AX	6.0	123,613
Screening - in vivo	L-2AX	14.0	288,431
Screening - in vivo	S-2AX	2.0	41,204
Related new assay development	E-2AX	10.0	206,023
Tumor cell kinetics - in vivo	E-2AX	6.0	123,613
Detailed drug evaluation - in vivo	L-2B2	18.0	370,840
Detailed drug evaluation - in vivo	S-2B2	19.0	391,442
Detailed drug evaluation - in vitro	S-2B2	1.0	20,602
Related new assay development	E-2B2	0.5	10,301
Detailed drug evaluation - in vitro - microbial	S-2B2	2.5	51,506
Develop. - Animal models of human tumors	E-2B2	21.0	432,647
SOUTHERN RESEARCH INSTITUTE 71-2189		100	27,420
Screening - Biochemical - new assay development	E-2AX	100	27,420

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project (Code)	(%)	($)
UPJOHN COMPANY 68-1023*		37.0	256,468 693,156
Bioassay - in vitro	L-1XX	8.0	55,452
Bioassay - in vivo	L-1XX	6.0	41,589
Screening - in vitro	L-2AX	2.0	13,863
Related new assay development	E-2AX	7.0	48,521
Screening - in vivo	L-2AX	4.0	27,726
Detailed drug evaluation - in vitro	S-2B2	10.0	69,316
WARF INSTITUTE 66-494		100	587,244
Bioassay - in vivo	L-1XX	37.5	220,217
Related assay development	E-1XX	2.0	11,745
Screening - in vivo	L-2AX	55.0	322,984
Related new assay development	E-2AX	0.5	2,936
Detailed drug evaluation - in vivo	L-2B2	5.0	29,362
WISCONSIN, UNIVERSITY OF 67-1126		100	15,995
Bioassay - in vitro	L-1XX	95.0	15,195
Related - new assay development	E-1XX	5.0	800
YALE, UNIVERSITY OF 71-2339		100	55,000
Biochemical tests aimed at new agent synthesis	E-1XX	100	55,000
YALE, UNIVERSITY OF 73-3711		67.0	16,080 24,000
Screening - Biochemical	S-2AX	67.0	16,080
CENTRAL DRUG RES.(INDIA) PL-480*		25.0	--
Bioassay - in vivo	L-1XX	15.0	
Screening - in vivo	L-2AX	10.0	

Table 2 (Cont'd.)
DEB Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Drug Evaluation Branch	Funding of Tasks Monitored by Drug Evaluation Branch Distribution by Project (Code)	(%)	($)
INDIAN CANCER RES.CENTRE PL-480		70.0	--
Bioassay - in vivo	L-1XX	20.0	
Screening - in vivo	L-2AX	50.0	
WEIZMANN INST. (ISRAEL) PL-480		50.0	--
Screening - Biochemical - Assay Develop.	E-2AX	50.0	
WEIZMANN INST. (ISRAEL) PL-480*		20.0	--
Screening - in vivo	L-2AX	20.0	

*Contract with Project Officer responsibility outside DEB. For all contracts involving projects that are not monitored by DEB, funding is shown as the amount monitored by DEB/total cost.

Table 3
DEB Projects. Distribution of Effort Among Contractors

PROJECT DESCRIPTION		CONTRACTOR (Contract Number)		FUNDING ($) (Negotiated FY '73 or Obligated FY '72)
BIOASSAY - IN VITRO			L-1XX	283,090
Natural product fractions (B numbers, D-K samples)	Microbial Assays	Bristol (35)	110,396	
		Upjohn (1023)	55,452	
			ST 165,848	
	Mammalian Cell Cultures	Little (3727)	35,033	
		Miami U. (2225)	904	
		Mich. DH (3702)	45,115	
		Res.Triangle (2019)	20,995	
		Wisc. U. (1126)	15,195	
			ST 117,242	
BIOASSAY - IN VITRO - NEW ASSAY DEVELOPMENT			E-1XX	12,478
		Little (3727)	11,678	
		Wisc. U. (1126)	800	
BIOASSAY - IN VIVO			L-1XX	660,671
Natural product fractions (B numbers, D-K samples)	Current Systems (L1210, P388)	BMI (2182)	472	
		Bristol (35)	156,275	
		Hazleton (3704)	97,734	
		IITRI (1021)	136,257	
		Little (3727)	7,007	
		Mason (2259)	1,120	
		Upjohn (1023)	41,589	
		WARF (494)	220,217	
BIOASSAY - IN VIVO - NEW ASSAY DEVELOPMENT			E-1XX	11,745
Fractionation methods eval.		WARF (494)	11,745	
BIOCHEMICAL TESTS AIMED AT NEW AGENT SYNTHESIS			E-1XX	78,500
		Scripps (2042)	23,500	
		Yale U. (2339)	55,000	

Table 3 (Cont'd.)
DEB Projects. Distribution of Effort Among Contractors

PROJECT DESCRIPTION		CONTRACTOR (Contract Number)	FUNDING ($) Negotiated FY '73 or Obligated FY '72)	
TUMOR BANK			L-1XX	9,342
(Prorated for L-1XX in vivo tests)		Little (3727)	9,342	
BIOASSAY and ENZYME PRODUCTION			L-1XX	20,495
(Purification of Glutaminase)		Delaware U. (2115)	20,495	
SCREENING - IN VITRO			L-2AX	333,623
Crude natural products (B numbers B001,2,3 samples Non-SAC synthetics	Microbial Assays	Bristol (35)	28,674	
		Upjohn (1023)	13,863	
			ST 42,537	
	Mammalian Cell Cultures	Little (3727)	35,033	
		Miami U. (2225)	128,241	
		Res. Triangle (2019)	4,199	
		South. Res. (2098)	123,613	
			ST 291,086	
SCREENING - IN VITRO - NEW ASSAY DEVELOPMENT			E-2AX	48,521
		Upjohn (1023)	48,521	
SCREENING - IN VIVO			L-2AX	3,309,329
Crude natural products (B numbers, B001,2,3 samples Non-SAC synthetics	Current Systems (L1210, P388)	BMI (2182)	367,407	
		Bristol (35)	58,782	
		Hazleton (3704)	390,936	
		IITRI (1021)	674,113	
		Mario Negri (3720)	99,354	
		Jap. Fd. C.R. (2054)	14,125	
		Little (3727)	420,398	
		Mason (2259)	383,511	
		MAI (3728)	261,562	

Table 3 (Cont'd.)
DEB Projects. Distribution of Effort Among Contractors

PROJECT DESCRIPTION		CONTRACTOR (Contract Number)	FUNDING ($) Negotiated FY '73 or Obligated FY '72)	
SCREENING - IN VIVO (Cont'd.)				
		South. Res. (2098)	288,431	
		Upjohn (1023)	27,726	
		WARF (494)	322,984	
SCREENING - IN VIVO			S-2AX	128,033
Non-SAC synthetics	Non-Current but established systems (B16,LL, W256,etc.)	Hazleton (3704)	13,962	
		Little (3727)	46,711	
		MAI (3728)	26,156	
		South. Res. (2098)	41,204	
SCREENING - IN VIVO - NEW ASSAY DEVELOPMENT			E-2AX	837,369
Agents of known activity used as tools	Model Screens Eval. etc.	BMI (2182)	18,866	
		Bristol (35)	25,807	
		Hazleton (3704)	13,962	
		IITRI (1021)	193,628	
		Little (3727)	200,857	
		Mason (2259)	12,317	
		MAI (3728)	162,973	
		South. Res. (2098)	206,023	
		WARF (494)	2,936	
TUMOR CELL KINETICS - IN VIVO			E-2AX	123,613
		South. Res. (2098)	123,613	
SCREENING - BIOCHEMICAL			S-2AX	16,080
Non-SAC Synthetics	Inhibitions of known predictive value for in vivo activity	Yale U. (3711)	16,080	

Table 3 (Cont'd.)
DEB Projects. Distribution of Effort Among Contractors

PROJECT DESCRIPTION		CONTRACTOR (Contract Number)	FUNDING ($) Negotiated FY '73 or Obligated FY '72)	
SCREENING - BIOCHEMICAL - NEW ASSAY DEVELOPMENT			E-2AX	32,956
		Penna. U. (2188)	5,536	
		South. Res. (2189)	27,420	
TUMOR BANK			L-2AX	23,366
(Prorated for L-2AX in vivo tests)		Little (3727)	23,366	
DETAILED DRUG EVALUATION - IN VITRO			S-2B2	207,977
Drugs of Clinical Interest	Cell Culture(CXT)	Little (3727)	56,053	
	Microbial (Res. & X-Res.)	South. Res. (2098)	51,506	
	Cell Cycle Stage	AEC (56)	10,500	
	Specificity and other in vitro	South. Res. (2098)	20,602	
		Upjohn (1023)	69,316	
DETAILED DRUG EVAL - IN VITRO - ASSAY DEVELOPMENT			E-2B2	25,472
	Cell Culture	Little (3727)	4,671	
	Cell Cycle Stage Spec.	AEC (56)	10,500	
		South. Res. (2098)	10,301	
DETAILED DRUG EVAL - BIOCHEMICAL MECHANISMS			S-2B2	325,536
Drugs of Clinical Interest	Inhibition of Selected Biochemical sites	Penna. U. (2188)	5,536	
		South. Res. (29)	320,000	
DETAILED DRUG EVALUATION - IN VIVO			L-2B2	1,427,420
SAC Agents with confirmed screening activity	Route Dependency	BMI (2182)	84,895	
	Sch. Dependency	Bristol (35)	7,169	
	Comb. Therapy	Hazleton (3704)	146,601	
	Established Tumor Assays(L1210,P388, L5178Y,B16,LL,W256)	IITRI (1021)	200,800	
		Little (3727)	303,621	
		Mason (2259)	123,171	

Table 3 (Cont'd.)
DEB Projects. Distribution of Effort Among Contractors

PROJECT DESCRIPTION		CONTRACTOR (Contract Number)	FUNDING ($) Negotiated FY '73 or Obligated FY '72)	
DETAILED DRUG EVALUATION - IN VIVO (Cont'd.)				
		MAI (3728)	160,961	
		South. Res. (2098)	370,840	
		WARF (494)	29,362	
DETAILED DRUG EVALUATION - IN VIVO			S-2B2	652,562
Drugs of Clinical Interest	Special Assays Brain tumors, spont. AKR, Host Defense Mech.etc.	Hazleton (3704)	34,905	
		Little (3727)	93,422	
		MAI (3728)	132,793	
		South. Res. (2098)	391,442	
DEVELOPMENT - ANIMAL MODELS OF HUMAN TUMORS			E-2B2	812,550
Drugs of Clinical Interest	Spont. and Early gen. mammary tumors; metastases; surgical-adjuvant	Cath. Med. (3703)	182,646	
		Little (3727)	116,777	
		MAI (3728)	80,480	
		South. Res. (2098)	432,647	
TUMOR BANK			L-2B2	14,013
(Prorated for L-2B2 in vivo tests)			14,013	

Table 4
DEB Intramural Laboratory Projects
(See Individual Project Reports)

Project	DCT Array Code	Pct. of total Effort	Objective
Serial No. 3576 DCT, DR&D, DEB Office of the Chief MAI - Contract 1283 Drug Evaluation Division	L-2AX	60	Rapid screening in vivo of contract synthesized analogs and direct reporting to synthesis contractor
	E-2AX	10	Development, improved screening assays, in vivo (Mini-screen)
	L-2B2	25	Detailed drug evaluation. (Schedule dependency-5%; combination chemotherapy-20%)
	S-2B2	5	Detailed drug evaluation. (Non-current in vivo systems)
Serial No. 3583 DCT, DR&D, DEB Office of the Chief	E-1XX	10	Development of new assays for natural product isolation based on cell surface binding
	E-2AX	30	Development of new assays for determining potential anti-tumor specificity in vitro
	S-2B2	20	Detailed drug evaluation; non-current systems
	E-2B2	40	Development of new assays for detailed drug evaluation; based on binding properties of drugs and effects on host defense mechanisms

Table 4 (Cont'd.)
DEB Intramural Laboratory Projects
(See Individual Project Reports)

Project	DCT Array Code	Pct. of Total Effort	Objective
Serial No. 3577 DCT, DR&D, DEB Immunotherapy and Immune Response Modifiers Section. MAI-Contract 1283 Immunology Division	S-2B2	35	Detailed drug evaluation in vitro and in vivo for immune suppression and immune enhancement; drug effectiveness against AKR lymphoma
	E-2B2	65	Development of model systems to measure immunosuppression and immunoenhancement; development of models to evaluate remission maintenance
Serial No. 3578 DCT, DR&D, DEB Screening Section	L-2AX	5	Screening in vivo of special agents with stability and solubility problems
	E-2AX	50	Development of new assays for in vivo screening
	L-2B2	25	Detailed drug evaluation in vivo; current systems
	S-2B2	10	Detailed drug evaluation in vivo; non-current systems
	E-2B2	10	Development of new assays for detailed drug evaluation

Table 5. Screening Activity* of Drugs with Established Effectiveness Against Human Solid Tumors

NSC No.	Name	IP L1210	IP P388	IP or SC B16	IM or SC LL
		(Increase in Lifespan over Controls, %)			
26271	Cyclophosphamide	>200	150	76	80
123127	Adriamycin	64	>200	187	N
740	Methotrexate	105	122	N	N
19893	5-Fluorouracil	80	120	40	N
79037	CCNU	>200	172	200	30
409962	BCNU	>200	>200	109	44
95441	Me-CCNU	>200	90	134	100
8806	Melphalan	120	200	105	N
762	Nitrogen mustard	60	200	85	N
13875	Hexamethyl melamine	N	N	N	N
27640	5-FUdR	52	155	38	28
6396	Thio-TEPA	80	31	N	30
49842	Vinblastine	40	112	120	N
67574	Vincristine	47	142	89	N
3088	Chlorambucil	31	98	32	N
125066	Bleomycin	N	50	66	58
3053	Actinomycin D	45	>175	103	N
26980	Mitomycin C	70	150	67	N
104800	Dibromodulcitol	52	N	N	30
3070	Stilbestrol	N	N	N	N

* Results for IP treatment. In most cases, treatments were given daily. For L1210, activity on the optimal schedule is listed for markedly schedule dependent drugs.

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Serial No. NCI-3576
1. DCT, DR&D
2. Drug Evaluation Branch (DEB)
3. Office of the Chief
4. Microbiological Associates, Inc. (MAI)
   Cancer Chemotherapy Department
   Drug Evaluation Division
   Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Detailed Evaluation of Potential Antitumor Drugs.

Previous Serial Number: Same

Principal Investigator: John M. Venditti

Co-Principal Investigators: I. Kline, R. D. Costlow, R. J. Woodman, M. Gang (MAI Contract Nos. 68-1283 (to 1/13/73), 73-3728 (Current))

Collaborating Units: Other Divisions within this contract; Office of the Associate Scientific Director for DR&D, A. Goldin) Screening Section, DEB (B. J. Abbott); DCT, Experimental Therapeutics (J.A.R. Mead)

The Drug Evaluation Division, MAI, is a collaborative laboratory resource for the Staff, DEB, which acts in an advisory capacity in the planning, execution, and evaluation of tasks.

Man Years (NIH): Total 1. Professional 1.

Project Description: This is a multifaceted project devoted to the rapid conduct of in vivo experiments and reporting of results of specially assigned tasks relative to the recognition, development, and more efficacious usage of antitumor drugs.

Objectives:

1. Development of systems for priority screening of drugs synthesized under other DR&D contracts and rapid submission of results to synthesis contractor to aid in new drug design.

2. Development of new in vivo screening models aimed at improving compound utilization and cost efficiency.

3. Detailed evaluation of active antitumor drugs with respect to treatment route and schedule dependency.

4. Achievement of therapeutic control of malignant disease in animals emphasizing the enhancement of selective toxicity to tumor cells with individual drugs and combination therapy.

Methods: Studies are conducted mainly in rodents with transplantable tumors using established procedures developed within this and other projects sponsored by DR&D, DCT.

Major Findings: Only selected findings of special interest are summarized herein. Detailed descriptions of accomplishments are contained in the annual and interim progress reports, contract 68-1283 and 73-3728.

Drugs discussed in this project report are listed below with their NSC numbers and the abbreviations used.

NSC No.	
740	Methotrexate (MTX)
742	Azaserine
749	8-Azaguanine
752	6-Thioguanine (6-TG)
755	6-Mercaptopurine (6-MP)
1026	Cyclopentanecarboxylic acid, 1-amino-
1895	Guanazole
3062	Dichloro-daraprim
4728	2-Amino-1,3,4-thiadiazole
19893	5-Fluorouracil (5-FU)
26271	Cyclophosphamide (Cytoxan)
27640	5-Fluorodeoxyuridine (5-FUdR)
32065	Hydroxyurea
32074	6-Azauridine
32946	Methyl-GAG (MeGAG)
33669	Emetine
37023	Serine, ester with (2-chloroethyl)carbamic acid, DL-(CB 3210)
45388	Imidazole-4-carboxamide,5-(3,3-dimethyl-1-triazeno)- (DTIC)
51143	1H-Pyrazolo [2,3-a] imidazole
63878	Cytosine Arabinoside (Ara-C)
67574	Vincristine (VCR)
69187	Methoxy-ellipticine
71795	Ellipticine
77213	Procarbazine
79037	CCNU
82151	Daunomycin
82196	Imidazole-4-carboxamide,5-[3,3-bis-(2-chloroethyl)-1-triazeno- (BIC)
83251	Deoxycytidine
83265	L-Alanine, 3-(tritylthio)-
85998	Streptozotocin

NSC No.	
95441	Methyl-CCNU (MeCCNU)
96349	Coralyne
100880	Camptothecin, Na salt (Camptothecin)
102627	1-Propanol,3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride (Methane sulfonate derivative)
102816	5-Azacytidine
104800	Dibromodulcitol (DBD)
107392	5-Hydroxypicolinaldehyde thiosemicarbazone (5-HP)
109724	Isophosphamide (Isoph)
113089	Quinoline, 6-amino-1-ethyl-4-[p-[[p-(4-pyridylamino)phenyl] carbamoyl]anilino]-bromide, ethobromide
113112	Phosphinic amide, P,P-bis(2,2-dimethyl-1-aziridinyl)-N-2-pyrimidinyl-
119875	cis-Platinum (II) diaminodichloride (cis-Pt-II)
123127	Adriamycin
125066	Bleomycin
126849	3-Deazauridine
129943	2,6-Piperazinedione,4,4'-propylenedi-, (+)- (ICRF-159)
132313	Galactitol,1,2:5,6-dianhydro-
135962	Cytosine arabinoside-palmitate (Palmo-Ara-C)
139490	5-Methyl-tetrahydro,-homofolic acid
145668	0-2,2'-Cyclocytidine, hydrochloride and the mon acetate (NSC-129220) (Anhydro-Ara-C)
409962	BCNU

I. Priority Screening of Compounds of Special Interest

This project was initiated in October 1972. It is designed to provide for the rapid specialized screening of compounds synthesized by DR&D screening contractors and the direct and rapid feedback of results to synthetic chemists to aid in the design of new agents for synthesis. On October 26, 1972, at a meeting of synthesis contractors, and DRD staff, agents being synthesized under contract were identified by structural type and specific protocols for screening of each type were designed. The various classes of agents so identified included selected nucleosides and analogs of VCR, Ellipticine, Daunomycin and Adriamycin, Streptozotocin, MTX, other antifols, Cytoxan, cis-Pt-II and other agents of sufficient interest to warrent contract projects for synthesis of congeners. Three principal features of the MAI special screening project are (a) the screening of specific analogs in assays of specific pertinence to the particular agent under study, (b) direct internal comparison of the new agent with the parent drug either in the clinic or in development to clinical trial, and (c) immediate submission of results directly to the synthetic chemist from MAI. It is expected that we will receive about 1350 contract synthesized compounds per year. Reports from contract-supported synthetic chemists and the Drug Development Branch, DR&D indicate that this project has been conducted most satisfactorily and we plan to continue it. Other "non-contract" generated analogs were compared for anti-L1210 activity. These included cis-Pt-II analogs and Ellipticine analogs.

II. Development of In Vivo Assays (L1210) for Screening of Materials Received in Small Amounts (The "Mini-Screen")

This laboratory was one of two selected to evaluate the feasibility of the model "mini-screen" for determining the presumptive activity of small amounts of new agents against leukemia L1210 in mice. The project involved the submission of 10 drugs of established anti-L1210 activity and inactivity in various amounts of 5.0, 10, 20, 40, 80, 100, and 150 mg. as unknowns. Thus, a total of 70 coded samples were screened. On February 7, 1973, the results reported from this laboratory and the other contract laboratory were evaluated considering (a) correlations with known effectiveness of the selected drugs, (b) intra-laboratory variability, and (c) inter-laboratory variability. The following conclusions were reached:

A. The mini-screen is feasible. Variability was low despite the small number of animals (3) per dose level. However, it should be noted that the model, as tested, utilized a greater number of dosage levels than required by the present DRD screening protocol for L1210. Also, measurement of local-subcutaneous tumor size and survival time were used as parameters of activity. Thus, the mini-screen, as tested, did affect a saving in drug utilization but no saving in total number of mice used or work effort.

B. Dose-response curves showed activity for known active compounds.

C. When the amount of material was < 20 mg., not all the true positives were selected. When the amount of material was ≥ 20 mg., most of the true positives were identified.

D. Inclusion of tumor size measurements increased the number of false positives. Use of survival time alone did not reduce the number of true positives.

E. Elimination of the tumor size measurement and reduction of the number of dosage levels in the dose response could reduce the cost of testing each drug. Thus, the "mini-screen" protocol, as tested in this project, would not reduce the cost of screening, but the development of a less expensive screen overall was not the object of this project. However, a suitable modification of the "mini-screen" might be used as a general primary screen and permit the primary screening of a larger number of materials without increasing total screening costs.

III. Combination Chemotherapy of Mouse Leukemia L1210

Sixty-four drug combinations were tested against early or advanced ip or sc-inoculated L1210 for therapeutic advantages over single drug treatment. A variety of dose-ratios, route and treatment regimens were used. Drug combinations providing an increase in lifespan (ILS) $\geq$ 50% greater than the ILS with the more effective individual drug were:

Adriamycin + ICRF-159, or DTIC
Ara-C + ICRF-159, BIC, or Procarbazine

DTIC + Camptothecin, or NSC-51143
Cytoxan + Coralyne, or NSC-113112
Anhydro-Ara-C + Procarbazine
5-Azacytidine + Emetine
Camptothecin + Isoph, or ICRF-159
ICRF-159 + NSC-113089

The previously reported synergistic combinations of ICRF-159 + Daunomycin, at an optimal dose of ICRF-159 plus a dose (ip) of Daunomycin which was lethal when used alone, was confirmed. The synergistic combination of ICRF-159 + Adriamycin on the other hand, generally consisted of a sub-optimal dose of Adriamycin plus an optimal dose of ICRF-159.

The superiority, reported previously, of early Adriamycin + DTIC therapy over individual drug treatment was confirmed and extended to show therapeutic synergism against advanced L1210.

Each of the 6 agents, NSC-1026, Procarbazine, MeCCNU, DBD, 5-HP, and Isoph, was studied in combination with either Cytoxan or Ara-C in mice with advanced L1210. None of these agents provided a superior therapy when given in combination with Cytoxan. In combination with Ara-C, Procarbazine and 5-HP provided an enhanced therapy over single drug treatment.

Treatment with 6-Azauridine protected the host against lethal doses of 5-Azacytidine. Although the maximally effective combination permitted the use of higher doses of 5-Azacytidine, the antileukemic effect was not superior to that achieved with lower doses of 5-Azacytidine alone.

Treatment with Deoxycytidine reduced the host toxicity and antileukemic effectiveness of Palmo-Ara-C. Deoxycytidine treatment reduced the host toxicity of higher doses of Anhydro-Ara-C without at the same time reducing the antileukemic effect of the lower doses. The maximum effect of the combination, although not superior to the maximum effect with Anhydro-Ara-C alone, was achieved over a wider range of doses.

IV. Route and Schedule Dependency of L1210 Leukemia

A. Schedule Dependency of Early IP L1210 Leukemia

Dichloro-daraprim displayed moderate activity on several ip treatment schedules; sc activity was retained but oral administration was ineffective. None of a variety of schedules used with Coralyne was superior to daily treatment; sc and oral treatment were ineffective. NSC-37023 tested over a wide range of doses on daily and intermittent treatment did not display toxicity or anti-L1210 activity.

B. Activity Against Early SC-Inoculated L1210

NSC-141540D (Discreet) and NSC-132313 displayed comparable activity whether given sc against ip L1210 or ip against sc L1210. Treatment with 5-HP given Q3H on every 4th day was clearly the superior schedule

and the antileukemic activity was comparable when this agent was injected either ip or sc in mice with either ip or sc implanted leukemia.

C. Activity Against Advanced SC-Inoculated L1210

Previous studies showed that the optimal schedule for Ara-C treatment of early L1210, Q3H on every 4th day, provided no advantage over a single treatment on 2nd or 4th day when treatment initiation was delayed until day 5. Four additional agents which are schedule dependent for treatment Q3H/4d in early L1210; Hydroxyurea, MeGAG, Guanazole, and 5-HP were tested against advanced sc L1210. Hydroxyurea and MeGAG were more effective given daily; Guanazole was more effective given daily or twice daily; 5-HP was only slightly more effective on the Q3H/4d schedule than via daily treatment. NSC-83265 and Emetine, schedule dependent for intermittent treatment in early L1210, did not display schedule dependency against advanced L1210. 5-Azacytidine displayed schedule dependency for daily treatment in early and advanced sc L1210.

V. Studies of Resistance and Cross-Resistance

5-Methyl-tetrahydro-homofolate was more effective against a number of MTX-resistant variants of L1210; L1210 DF-8, M-46R, M-66R with activity being observed over a wide range of doses including relatively low doses that have marginal activity against the L1210 sensitive subline. The agent was also effective against the L1210-DF-8 tumor inoculated ic. Studies with other types of tumors resistant to MTX are planned. (With J. A. R. Mead).

An Anhydro-Ara-C resistant subline of L1210 was developed. It was completely resistant to Ara-C. The resistant subline retained sensitivity to MTX, 8-Azaguanine, 6-TG, 6-MP, 5-FU, Cytoxan, Azaserine, NSC-1026, BCNU, Camptothecin, and Ellipticine. 3-Deazauridine (126849) displayed considerably more activity against the resistant subline as compared with parent L1210 line. The response of the Anhydro-Ara-C and Ara-C resistant sublines to treatment is being compared. (With J. A. R. Mead).

It was previously reported that a Cytoxan resistant subline of L1210 was cross-resistant to BCNU. It is therefore of particular interest that a subline developed for resistance to BCNU responded to Cytoxan treatment. L1210/BCNU was also sensitive to Ara-C, MTX, 6-MP and ICRF-159.

A dimethanesulfonate (NSC-102627) resistant subline was developed and tests for cross-resistance to other alkylating agents in progress.

VI. Studies with Lewis Lung (LL) Carcinoma

A. Single drug treatment

Four agents produced a survival time increase $\geq$ 50% when given IP daily to mice with IM inoculated LL. These were Cytoxan, Isoph, NSC-4728, and Bleomycin. Of these, all except Bleomycin are active

against L1210 leukemia. Twenty-six agents previously noted as active against KB cells in culture only were tested against LL. Six produced increases in survival time ≧ 25%.

B. Schedule Dependency

Schedule dependency studies were conducted with four agents with demonstrated activity against LL. As was previously demonstrated in the advanced L1210 assay, a single treatment with Ara-C on every 2nd or 4th day was superior to divided treatments on these days. Intermittent treatment until day 17 was generally superior to treatment limited to 9 days. NSC-4728 displayed comparable activity whether given daily or every 4th day against early LL. 5-FUdR was effective against early LL tumor on several treatment schedules but the maximum increase in survival was achieved on the Q2d, days 1-17 schedule. Isoph was studied on a number of treatment schedules (day 1 thru 9 and days 1 thru 17), and none of the schedules was superior to a single treatment on day 1 only.

Against early LL, a single treatment with Cytoxan on day 1 produced "cures". Against established LL, a single treatment on day 6, while superior to 2 weekly or 7 daily treatments, did not result in "cures". Although treatment Q6h on day 6 only elicited the maximum survival, repeated treatment in divided doses every 4, 8, or 12 days was inferior to treatment with a single injection on the same days.

C. Combination Chemotherapy of LL

Bleomycin was given in combination with each of 8 agents in the treatment of early LL. Combined therapy with either CCNU, VCR, or Procarbazine was superior to single drug treatment. Against established LL, enhancement over single drug therapy was confirmed for the combinations of Cytoxan plus ICRF-159 and Bleomycin plus Procarbazine. Studies to confirm other indicated advantageous combinations with Bleomycin are in progress.

D. Influence of Chemical Agents on LL Metastasis

Utilizing the Wexler India ink assay, Bleomycin, Isoph, and Cytoxan were shown to inhibit LL lung metastases as well as to inhibit primary growth and increase the lifespan of tumor-bearing mice.

E. Growth of LL in Mice Pretreated in Cytoxan or X-irradiation

X-irradiation of mice prior to tumor inoculation reduced the number of LL cells necessary for tumor production. However, pretreatment with X-irradiation or Cytoxan prior to inoculation of LL cells appeared to retard the growth of the local tumor. Pretreatment with Cytoxan prior to implantation of LL cells appeared to enhance metastatic growth.

Proposed Course:

Since initiating the rapid screening and direct reporting of results with contractor-synthesized analogs, this activity has utilized 60% of the total effort of this project. Because of its success, this task will be continued. However, it is anticipated that detailed drug evaluation (schedule and route dependency, and combination therapy) will continue to be emphasized. Routine primary screening other than for contractor-synthesized agent testing, will be largely eliminated. Lewis lung testing primary screening has been eliminated and the system will be used selectively as a secondary screen for drugs of special DCT-program interest.

Publications:

See current report, Drug Evaluation Branch, Office of the Chief.

BIOCHEMISTRY SECTION

DRUG EVALUATION BRANCH

DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The ultimate objective of the Biochemistry Section program is the improved therapy of human cancer. The program is implemented through contracts with qualified institutions. This report summarizes the activities and major findings of each contract. Approximately one third of the total effort falls under each of the DCT program arrays; linear, supplementary, and exploratory. The detailed breakdown of effort relative to specific segments of the DCT array is shown in the current Organization Report of the Drug Evaluation Branch.

SCRIPPS CLINIC AND RESEARCH FOUNDATION, CONTRACT NCI-72-2042.

This contract was initiated to prepare and evaluate vitamin B_{12} analogs. This vitamin is known to be essential for cell division and maturation in man, and to be obtained entirely from exogenous sources. Hence if such antagonists, particularly of the active coenzyme form of the vitamin, have the ability to inhibit the replication of cells in culture or in host animals, they would be potentially useful agents for chemotherapy of cancer in man. Chemical procedures for the synthesis of agents of the cobalamin series (alteration of the upper ligand by a false base or an alkyl-group, but no change in lower 5,6-dimethylbenzimidazole ligand) have been worked out to give overall yields of 50%—70%. Twelve agents have so far been prepared. However, while synthesis has turned out to be unexpectedly easy, biological evaluation, due in part to gaps in basic knowledge of the detailed metabolic role of this vitamin, has presented more complexities. Assay of the agents as inhibitors of ribonucleotide reductase from Lactobacillus leichmannii, known to require B_{12}, has been straightforward. Two of the agents, AraA-B_{12} and formycinyl-B_{12}, were found to have sufficiently good K_i values to make them candidates for antitumor testing. First evaluation of the alkyl cobalamin analogs is to be by methionine synthetase inhibition, since this enzyme requires methyl-B_{12}. However, the required preparation of the apoenzyme has not yet been accomplished. However, the following tentative conclusions have been reached from the enzymatic results to date: (1) that B_{12} enzyme systems have rather rigid structural requirements with respect to both the purine (or pyrimidine) and the sugar moieties and that analogs whose structures are quite different from adenosyl-B_{12} are not likely to be effective inhibitors; and (2) that because most, if not all, of the B_{12} systems in the cell have tightly bound coenzyme, analogs are not likely to displace the latter. However, analogs would be expected to become increasingly effective over a period of several cell divisions which would allow them to compete more favorably with adenosyl- and methyl-B_{12} for the coenzyme site on newly-synthesized protein.

The transport of B_{12} into karyotic cells is known to require the aid of a serum protein, transcobalamin II (TC II). There is no data on the transport requirements of the analogs, or of competition with natural serum B_{12} etc. To clarify these points, TC II, purified about 65,000-fold, has been prepared to allow

studies with ^{57}Co-labeled materials to be done.

The uptake of ^{57}Co-cyanocobalamin into L1210 cells in culture has been shown to follow a biphasic pattern with time and to be absolutely dependent on added TC II.

SOUTHERN RESEARCH INSTITUTE, CONTRACT PH-43-66-29.

Work under this contract includes a broad array of individual projects which will, for the sake of clarity, be grouped under four main headings: 1) Studies of the nitrosoureas, 2) Studies of mechanisms of action of purine and pyrimidine anticancer agents, 3) Studies of some basic pathways in nucleic acid metabolism in order to identify and define the most effective use of inhibitors of them, and 4) Miscellaneous studies. The accomplishments in each area follows.

1) Nitrosoureas. As a summary of the many years of study of these agents the following conclusions were reached: a) The shorter the half-life the greater the alkylating and less the carbamoylating activities, b) The combined activities correlate better than either activity alone with in vitro and in vivo toxicity and with antitumor activity, c) As carbamoylating activity becomes a greater part of the combined activities the therapeutic index worsens; thus the "best" nitrosoureas should have the minimum of carbamoylating potential to insure lipid solubility, and d) That normal tissues have a faster recovery from nitrosourea treatment than tumors, a fact that may have clinical applications.

2) Mechanisms of action of purines and pyrimidines.

a) Deazauridine (NSC-126849). This agent, although Roswell-Park finds it very active in L1210, gives a maximum T/C of ~ 150 on daily treatment of 225 mg/kg/day in our L1210. In L1210/AraC, which is resistant because of lack of deoxycytidine kinase, daily treatments of 40-60 mg/kg/day cure about half the mice. In vitro studies by this contract showed the growth of Ca755 cells to be inhibited by this agent. Uridine, cytidine, and particularly deoxycytidine reversed the growth inhibition while thymidine and deoxyuridine did not. Incorporation of precursors (C^{14}-formate and adenine-8-C^{14}) into DNA was inhibited by deazauridine but was restored to normal with the combination of deazauridine and deoxycytidine. All of these facts have led to the present hypothesis, which is that deazauridine interferes with the formation of pyrimidine deoxyribonucleotides. The possible protection from deazauridine toxicity and enhancement of antitumor activity in vivo by deoxycytidine is under investigation with Dr. W. R. Laster Jr. of SRI contract NIH-71-2098.

b) 6-Methylmercaptopurine riboside (NSC-40774). In the hopes of establishing a "rescue" type use of this drug (comparable to the MTX-citrovorum factor) attempts were made to reverse the toxicity of NSC-40774 in normal mice by AIC or hypoxanthine. If the primary site of action is inhibition of de novo purine synthesis, as had been accepted from previous work, reversal would have been accomplished. However, no indications of reversal were obtained. Possible explanations are i) that multiple sites of action, or different sites, are involved in toxicity, or ii) that the reversing agents do not reach the critically-affected cells in sufficient quantity to produce reversal.

c) 8-Azainosine (NSC-130279) and 8-azaadenosine (NSC-72961). Studies

on these compounds were undertaken some years ago because these agents were found to be active against cells resistant to azahypoxanthine. Experiments during this year were related to i) defining the active metabolite, ii) attempting to define the mechanism of resistance to azainosine, and iii) study of effects of the agents in purine and pyrimidine metabolism. 8-aza IMP, or a further metabolite, seems to be the toxic metabolite of both agents, but, after elimination of all the usual points of purine analog activity, the site of its action remains undefined.

3) Studies of nucleic acid metabolic pathways, and the identification of inhibitors.

a) Ribonucleotide reductase. Perhaps the most potentially useful work to come from this contract this year has resulted from further study of the increased effectiveness and decreased toxicity of ribonucleotide reductase inhibitors (hydroxyurea, guanazole, thiosemicarbazones) by the simultaneous use of Desferal. This agent, because it is a strong chelator of iron salts, is used clinically in iron poisoning. With a thiosemicarbazone (NSC-107392, IND 6557) and Desferal more than half of groups of L1210-inoculated mice were cured. Since both agents are clinical drugs, use of this finding could be made without delay.

b) Repair of DNA damage. So far, after several years of study, a satisfactory, quantitative, reproducible method for inflicting DNA damage has not been worked out. Previously discarded were x-rays and alkylating agents. This year the use of bleomycin proved to be no better.

4) Miscellaneous.

i) SRI has a battery of H.Ep.2 cell lines which are resistant to various purine analogs by reason of clearly defined enzyme deletions. The mechanism of action of any different purine analog can be fairly well defined by its effects in the sensitive and these resistant lines. Hence, small quantities of all new, in vivo active purines are to be so studied. Work on this is just getting underway.

ii) A preliminary survey of area of mechanism of action of selected agents with unique structures and confirmed in vivo activity has been undertaken. The approach is to see whether there is pronounced inhibition of incorporation of labeled TdR, UR or Leucine into DNA, RNA or protein by the agent. Where such activity is found, it is planned to identify the site of action at the molecular level. So far seven agents have been scanned, with three showing activity of interest.

SOUTHERN RESEARCH INSTITUTE, CONTRACT NIH-71-2189.

The objective of this contract is to establish whether there are anticancer agents which exert cytotoxic action primarily as a result of inhibition of membrane formation. The approach chosen was to determine in vitro the effect of an agent on cell growth and on incorporation of labeled precursors into membrane constituents and DNA. The most-studied precursor for membranes has been choline, with lesser effort to date on glucosamine, serine, acetate, inositol, etc. While Ca755 cells were used initially, they were rather soon replaced by L1210 cells. About fifty agents have been evaluated, of which eight

have exhibited preferential inhibition of one or more membrane precursor over TdR into DNA or over cell growth.

The real question in determining the value of this contract is what the level of preferential inhibition (membrane over cell replication) must be when this becomes the prime cause of cell stasis or death; or, indeed, whether such an inhibitory mechanism can in itself be lethal. To attempt an answer to this basic question, the Drug Development Branch was asked to prepare tetrolyl-pantetheine (NSC-169437) and four variations (NSC-166364, 166906, 167679 and 169436). NSC-169437 was found by Dr. R. O. Brady to inhibit the incorporation of acetate-1-^{14}C into the lipids of rat skin by 70%. The "Brady compound" itself (NSC-169437) was most difficult to isolate and we have received only a small amount. However the requested amount of NSC-167636, which was much easier to prepare, and which differs from NSC-167637 only in the absence of two hydroxyl groups, has come. It is hoped that full evaluation of these agents will afford some clarification in the near future of the basic issue of the usefulness of the present approach.

UNIVERSITY OF PENNSYLVANIA, CONTRACT NIH-71-2188.

The purpose of this contract is to explore the possibility that useful anti-cancer agents may have significant effects on the energy-producing functions of cells, and, if such is found to be the case, to establish structural requirements for this action. During the past year agents of a number of different classes were studied. These included DR&D agents such as daunomycin, ICRF159, CCNU, MeCCNU, diaminochloroplatinum, picolinaldehyde thiosemicarbazone, ethidium bromide, hydroxyurea, etc. Their effects on respiration of Ehrlich cells *in vitro* and, where inhibition was obtained, on uncoupling of oxidative phosphorylation and on electron transport were determined. CCNU and MeCCNU were good inhibitors of respiration, with significant inhibition of both uncoupling and electron transport, but at fairly high levels of drug.

The changes brought about by these drugs on oxygen consumption of intact, isolated mitochondria from rat liver and pigeon heart in state 4 (in the presence of substrate and P_i, but in the absence of ADP; respiration is very low) and state 3 (as in state 4, but in the presence of ADP; respiration is increased) were also determined. Emetine, ICRF159, CCNU, MeCCNU, diaminochloroplatinum, and ethidium bromide showed inhibition in state 3 and daunomycin, hydroxyurea, the thiosemicarbazone, and ethidium bromide increased oxygen consumption in state 4. To daunomycin and ethidium bromide in particular, the pigeon heart muscle mitochondria were more responsive than those from rat liver. Work now underway will clarify whether this is a tissue or species difference.

To sum up the work so far, one can say that various anticancer drugs do have differential effects at various places in the energy-producing system in cancer and normal cells. The work at this time is not in a state of completeness to come to any conclusions about the usefulness of effects in this area as a anti-cancer screen. Further and more tightly targeted work (on L1210 cells and their mitochondria, etc.) should provide an answer.

WEIZMANN INSTITUTE OF SCIENCE, PL480 AGREEMENT NIH-06-025-1.

The work under this agreement is the study of the physical-chemical properties of polymers as they relate to biological activity. The objective is to either develop new anticancer agents or to modify the effects of known agents to achieve greater utility. Changed physiological distribution, release, metabolism, etc. could be the result of such modification.

Toward the end of last year, eight derivatives of melphalan were received for testing (NSC-154852-154858). In comparative testing with melphalan (NSC-8806) against intraperitoneal and intracranial P388, activity was not increased by any modification. Full activity was retained by three or four of the agents.

A group of fourteen low molecular weight polymers (~ 30 units) of essentially leucine and orinthine or derivatives of each have just been received (NSC-169842-169855). Since these agents would, on the basis of molecular size, not be expected to get into cells, plans are underway to test them a) for antitumor activity in the mini-screen, b) as modifiers of antigenic properties, and c) as interferon inducers.

YALE UNIVERSITY SCHOOL OF MEDICINE CONTRACT NIH-71-2339.

Work under this contract started with a study of acridine and analogs of it as a model for agents which might prevent nucleic acid synthesis, and hence cell replication, not by an effect on polymerases or accumulation of the four deoxynucleoside triphosphates, but by preventing template separation. The measurements made in studying such a model have been tightness of binding to DNA (binding constant) and changes in DNA melting temperature (Tm, or a determination energy required for strand separation) by the agent.

Actinomycin D has 10,000 times the DNA binding capacity of the actinocin ring; the pentapeptide side chains ensure tight intercalation by forming hydrogen-bound pairs with adjacent nucleic acid bases. The acridine structure by itself, without such stabilizing groups, produces a greater effect on Tm than Actinomycin D. The binding constant of acridine was found last year to be subject to change by structural modifications of the molecule. Based on the previous experience and knowledge by the investigator of the physical-chemical relationships of the naturally-occurring polyamines and DNA, a series of agents were prepared in which the extreme ends of putrescine, spermidine or spermine were linked through the 9-NH_2 positions of two acridines.

These so called "double intercalators" have been partially evaluated physically and biologically. What is clear so far is that the binding constant and the Tm have been increased, the latter dramatically. Biologically, very preliminary results show that these agents a) interact most effectively with DNA, b) they react strongly with guanylic acid to yield a crystalline precipitate, c) they are incorporated into the nucleus, d) they seem to eliminate the nucleolus (as seen by electron microscopy), and e) they inhibit the production of some viruses. In summary, the series of agents very effectively interfere with "naked" or the nonprotein-associated form of DNA such as occurrs during S phase,

or in the inhibition of mRNA synthesis during other stages of the cell cycle.

YALE UNIVERSITY SCHOOL OF MEDICINE, CONTRACT NIH-73-3711.

During this year, a number of potential antifols have been evaluated as inhibitors of human leukemia dihydrofolate reductase, but none has shown activity of an interesting level. Most of the effort, however, has gone into preclinical study of the Baker antifol, NSC-139105 which is a candidate for clinical trial. The following are the findings in brief on this agent: CSF levels of NSC-139105 in the dog reached 10% to 20% of serum concentrations after i.v. injection. Following i.p. treatment with 36 mg/m^2 in the mouse and the rat, dihydrofolate reductase in liver, kidney, spleen, and brain was reduced to barely detectable levels by 15 minutes. Incorporation of ^{3}HUdR into DNA by W256 cells in culture was 95% inhibited by $2x10^{-6}$M NSC-139105, while DNA synthesis in L1210 cells was inhibited by only 45%. The basis for this differential effect was studied using extracts of the two cell types. Inhibition of dihydrofolate reductase from both cell types was marked ($I_{50} \cong 1.5x10^{-9}$M for W256 and $0.75x10^{-9}$ for L1210), and the drug was not metabolized by either cell type. However, when drug transport was studied in cell suspensions, the agent was found to accumulate to levels 50 times greater in W256 cells than in L1210 cells, indicating that this transport difference is probably the basis for the effectiveness of the compound in the W256 compared to the L1210.

FUTURE PLANS.

There are two different areas where the needs of the program which can be met by biochemical studies. They are:

1) To learn the mechanism of action of each of the agents in clinical trial or under development toward the clinic. Such knowledge may in some cases lead to decreased or circumvented toxicity, may suggest possible favorable drug combinations, or the selection of patients or tumors to receive the particular drug.

During the coming year it is planned to compete our one present source of these studies. It is to be hoped that among responding organizations there will be many well qualified to do these studies, and that we may expand our efforts to the point where all candidate agents can be studied.

2) The second area of biochemical application should be exploration of the feasibility of productive studies related to the relationships of plasma membranes to cellular metabolism and function. It is well known that the cellular membrane is all-important in immune responses, that it exerts a significant effect on regulation of the internal millieu of the cell through transport mechanisms. But it can well be that there are other functions about which the present state of knowledge is sufficient that reasonably well defined studies would be expected to yield information of value to chemotherapy.

Serial No. NCI-3577

1. Division of Cancer Treatment
   DR&D
2. Drug Evaluation Branch
3. Immunotherapy and Immune
   Response Modifiers Section
4. Microbiological Associates, Inc.
   (MAI), Immunology Division
   Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Transplantation, Serological, Chemotherapeutic and Immunochemotherapeutic Studies with Virus Induced Murine Leukemias and Sarcoma

Previous Serial No.: Same

Principal Investigator: D. P. Houchens

Other Investigators: A. Goldin (Principal Advisor)

Cooperating Units: Office of the Chief, Drug Evaluation Branch (J.M. Venditti); Office of the Associate Scientific Director, DR&D (A. Goldin and R. Johnson); Biology Branch, NCI (B. Zbar); MAI Contract PH-43-68-1283 (M. Kende)

Man Years (NIH):

Total: 2
Professional: 1
Other: 1
Plus animal care support from Mammalian Genetics and Animal Production Section, DRD.

Project Description:

Objectives:

1. To determine the efficacy of utilizing immunological reactions directed against tumor-associated antigens, through active immunotherapy or adoptive immunotherapy, in producing anti-tumor activity in murine viral-induced tumors.

2. To follow the cell-mediated and humoral immune reaction involved in the immunotherapy of murine tumor systems, with in vitro or in vivo assays.

3. To investigate the interactions between anti-tumor drugs and the host's

immune defense.

4. To utilize spontaneous and induced primary murine leukemias as models for developing chemotherapy, immunotherapy, and anti-viral regimens either singly or in combination and to develop assays to monitor the efficacy of these regimens.

Methods Employed: Standard Laboratory procedures were employed.

Major Findings:

I. Immune Response Modification

A. MSV. Studies have continued on the use of non-specific active immunotherapy of tumors with Bacillus Calmette-Guerin (BCG). As seen in the last annual report, BCG treatment of animals before immunosuppression markedly reduced the immunosuppressive effects in MSV infected BALB/c mice. These findings are reported in an article by Houchens, Goldberg, Gaston, Kende, and Goldin in Cancer Research, April 1973. Several experiments have been set up to evaluate the effects of BCG administration after immunosuppression and MSV inoculation. While the results are not as clear as in the pre-inoculation studies, there is some indication that BCG, post-treatment prolongs the median survival time of immunosuppressed animals which have received MSV as compared to animals which have not received BCG.

The MSV-BCG-Cytoxan systems is being studied further by use of the in vitro ^{51}Cr release cytotoxicity assay. Both the cellular and humoral responses of the mice are being monitored by this test and the data compared to the tumor growth. CBF_1 mice are being used for these tests instead of BALB/c mice because they have a better response as measured by this assay (Lavrin et al.) It was expected that the mice receiving BCG, CY, and MSV would have a higher percentage of cellular cytotoxicity than other groups since they had the smallest tumors. On the contrary, they generally had the least percentage of cytotoxicity. This indicates that the reason for tumor size reduction may be due to a suppression of blocking antibody (by the CY) more than to an increase of the cellular response.

To further study the effects of immune modification in this system four groups of BALB/c mice were given 1) BCG, CY, MSV; 2) BCG, MSV; 3) CY, MSV; 4) MSV; and a fifth group was untreated. Eight animals from each group were challenged with 10^4 LSTRA tumor cells (same antigens as MSV) on Day 7, 14, 21, and 35 post MSV. The survival curves showed that BCG plus MSV conferred the best protection while CY plus MSV decreased the MST to near that of the control animals. In the groups receiving BCG prior to CY and MSV, the MST was increased indicating that the immuno-suppressive effects were overcome. These findings are in agreement with those reported in the Cancer Research article mentioned above.

Experiments are in progress to determine the effect of dose response

of BCG in the MSV system since some workers have reported enhancement as well as suppression of various tumors. Early results indicated that high doses of BCG (10 mg[1 x 10^8] and 5 mg [5 x 10^7]) do enhance maximum tumor size.

B. LSTRA. BCG showed no effect against 10^2, 10^3, 10^4, or 10^5 LSTRA challenge. Tumor takes were higher in pretreated (22 days) mice than in controls.

Methanol extracted residue (MER) from BCG gave a slight but not significant increase in MST against LSTRA.

C. Tranplantable AKR Lymphoma. Normal AKR mice were injected with 1 or 5 mg. of BCG at various times before challenge with graded doses of transplanted AKR lymphoma. When treatment was given 7 days before challenge, the lifespan was increased with the highest ILS at the 10^3 challenge level (ILS >168%). The tumor sizes of the local tumors were smaller than the controls in the lower challenge levels (10^3, 10^4).

D. Lewis Lung. Tumor size reduction with non-living cell wall preparations (Ribi vaccine) of BCG has been reported by Zbar and others. This type of preparation does not have the disadvantage of growing in the host and infecting vital organs, yet the immune system is stimulated. C57Bl/6 mice were given graded doses of Lewis Lung tumor cells by the id route (with R. Johnson, Office of the Associate Scientific Director, and B. Zbar, NCI, Biology Branch). In some groups the tumor cells were mixed with BCG or Ribi cell wall preparation while other groups were treated at the tumor site several days later. The only cures occurred in groups where BCG or cell wall were mixed with tumor and injected. Established tumors were not affected by treatment. Further experiments are in progress in which mice are immunized by the iv route and given tumor cell suspensions by the same route 2-3 weeks later. The production of granulomas by BCG in the lungs should offer some protection against Lewis Lung tumor growth. (For further information, see report of R. Johnson, Office of the Associate Scientific Director, DR&D).

II. Chemotherapy of Murine Viral Induced Tumors

A. AKR Spontaneous Lymphoma

This system has continued to serve as a model for chemotherapy screening of antitumor agents and to find drugs capable of maintaining remission.

1) Single drug therapy

Of the compounds tested, this year the following ones produced greater than 25% ILS: MTX, Thioguanine, 6-MP, IUDR, Vinblastine, Rifampicin, BUDR, and Benzyl Rifampicin.

2) Combination drug therapy

Further studies have been carried out with combinations of drugs against spontaneous AKR Lymphoma. Combinations of MTX and prednisone were not as effective as either drug by itself. Combination of Thioguanine (1 mg/kg/injection) and prednisone (30 mg/kg/injection) gave ILS in the range of 50-60%. The Thioguanine alone gave 40% while the prednisone had no effect.

Various combinations of Nitrogen Mustard, Vincristine, Prednisone, and Procarbazine,(MOPP) were tested in the AKR system. Three different schedule combinations in which Prednisone was either 15 or 30 mg/kg/injection and the Vincristine was either 0.125 or 0.25 mg/kg/injection gave ILS of 33-59%. In one experiment the drugs were tested singly, in pairs, threes, or combined. The best effects were seen with: (a) Mustard (0.25 mg/kg/injection), Vincristine (0.25), Procarbazine (25.0), ILS 93%; (b) Nitrogen Mustard (0.25), Procarbazine (25.0), ILS 93%; (c) Vincristine (0.25), Procarbazine (25.0), ILS 107%.

3) Remission induction-maintenance

Several of the rifamycin derivatives were tested in the two-stage protocols for remission maintenance after remission induction with Vincristine and Prednisone. Only benzyl-dimethyl rifampicin (NSC-143,414) had any effect (50 mg/kg everyday for 8 days gave 36% ILS).

Both Freeze-dried (FD) and live (L) BCG were used for remission maintenance studies as reported in an abstract to be presented at the AACR meetings in April 1973 (Kende, Airall, Houchens, and Goldin). Neither preparation was effective without first inducing remission. FD-BCG increased the lifespan by 70 and 85% with 2 x 10^6 and 8 x 10^6 organisms/mouse respectively after Vincristine-prednisone while L-BCG gave a 67% ILS with 1 mg/mouse (approximately 1 x 10^7 organisms).

B. Primary Moloney Leukemia

The report of the effects of rifamycin derivatives in this system was made last year. Freeze-dried BCG has been tested and found to give an ILS of 20%. BUDR was also tested but found to be ineffective.

III. Antiviral compound testing

Interest has continued in the anti-viral effects of various compounds. Distamycin A (NSC-82150) showed a significant inhibitory effect on MLV in vitro with a 15-fold inhibition of plaques using 10 µg/ml drug for 6 days in the culture medium. There was only a 2-fold inhibition against MSV at this concentration of the drug.

Virazole, NSC-163039, has been reported to have a wide spectrum of anti-viral activity. It was found to have marked inhibitory effects that were dose related against both MLV and MSV in vitro. It has been tested in vivo against AKR spontaneous lymphoma with no effect and is presently being tested in MLV infected BALB/c mice.

Four of the Streptovaracin derivatives:

A NSC-48,810
C NSC-19,990
D NSC-156,216
E NSC-156,217

have been tested in vitro and in vivo against MSV.

Streptovaracin E (50 μg/ml) produced complete inhibition of foci in vitro whereas A, C, and D had no effect. Early results from the in vivo testing indicate that Streptovaracin A (200 mg/kg 2X) has marked inhibitory effect on the virus as measured by tumor size. Streptovaracin C also produced some tumor size reduction at 400 mg/kg. Interestingly, the highest dose of compound E (400 mg/kg) enhanced the tumor size while lower doses had no effect on tumor size.

IV. Clinical Biochemistry in Spontaneous AKR Lymphoma

Studies were conducted to determine whether changes occur in the blood chemistry of leukemic AKR mice that could correlate with the clinical condition.

The most significant changes were seen in elevation of LDH and SGOT activities in leukemic AKR mice (4-5 times over normal AKR mice). This would indicate damage to liver, lung, and heart tissue. The alkaline phosphatase levels were also higher (4 times) in the leukemic mice. These studies will continue to observe effects of drug treatment on the various blood chemistries.

Significance:

The purpose of these studies is to obtain a better understanding of the nature and biology of tumor associated antigens, the immunological response elicited in the tumor bearing host, the effect of chemotherapeutic agents on these responses, and the contribution that immune phenomena might make to the overall therapeutic effect. The knowledge derived from such studies can be applied to model systems with transplantable and primary tumors in mice involving immuno-therapy and immuno-chemotherapy, and this may lay the framework for subsequent clinical studies. Also, studies involving the possible anti-viral properties of certain chemotherapeutic agents should contribute to the use of these drugs in the treatment of human neoplasia.

Proposed Course:

This section will continue a 3-pronged attack on murine viral-induced tumors. Immunotherapy studies will probe more efficacious methods of using active and adoptive regimens and more in depth work with assays to determine the kinetics of the immune response to tumor antigens. Chemotherapy will involve study with the AKR Lymphoma and BALB/c primary Lymphoma in terms of observing activity of known clinically active agents and searching for new active agents. Blood chemistry in AKR will be examined, also. Anti-viral agents will be evaluated *in vivo* and *in vitro* to screen for activity and to distinguish true anti-viral effects from cytotoxicity. Eventually it is hoped immunochemo- and anti-viral therapy will be used in combination to achieve a highly effective antitumor regimen.

Publications:

See current Organization Report, Office of the Chief, Drug Evaluation Branch.

SCREENING SECTION

DRUG EVALUATION BRANCH

Objectives and Significance to the National Cancer Institute

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products in vivo, and to a limited degree, in vitro; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-depth evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment (DCT) Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions and through intramural laboratory studies. Activity against experimental tumors in vivo is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage. In vitro activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for in vivo screening, for following fractionation and isolation of active components of crude natural products with correlative in vivo activity, for providing information relative to mechanisms of drug action, and for developing assays for estimating effective drug levels in blood and tissues.

I. Staff Functions

A. General

The Screening Section is staffed by six senior professional and two junior professionals, one on detail from the Office of the Chief, DEB. Principal investigators are: B. J. Abbott (Head); D. Abraham (CO); M. C. Baratta (CO); R. I. Geran; N. H. Greenberg and M. M. Macdonald. Staff is supported by five technical and clerical personnel and two part-time biological aids. Staff serves as Project Officers for five contracts devoted predominantly to presumptive in vivo screening; Battelle Memorial Institute (71-2182), Hazleton Laboratories (72-3704), Illinois Institute of Technology (68-1021), Mason Research Institute (71-2259), and WARF Institute (66-494) and two contracts for in vitro screening methodology; University of Miami (70-2225) and University of Wisconsin (67-1126). A contract was initiated with the Catholic Medical Center (72-3703) for the evaluation of a mouse mammary carcinoma as an organ directed human tumor model. Staff, in collaboration with the Chief, DEB; Chief, DDB; Associate Scientific Director, DR&D; and Director, Division of Cancer Treatment (DCT), monitored in vivo and in vitro screening, secondary drug evaluation, and methodology tasks

performed under multifaceted contracts with Southern Research Institute (71-2098), Arthur D. Little, Inc. (73-3727), Bristol Laboratories (69-35), and Microbiological Associates, Inc. (73-3728). In reviewing biological effects of natural product fractions the Screening Section established directions of effort under additional DR&D contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories as indicated; coordinate total effort with other DR&D and DCT projects; design assays for selection of new active drugs, investigate usefulness and feasibility of new assays; recommend candidate drugs for clinical trial; and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Experimental Therapeutics Area, Clinical Trials Area, and for DCT Decision Network review.

B. Specific

In conduct of duties staff (1) review proposals for institution or renewal of contracts, recommend new projects to Chief, DEB and prepare materials for Program and contract reviewing bodies; (2) provide guidance and instruction to contract laboratories, monitor scientific and technical sufficiency, and indicate areas needing modification or clarification through frequent communication and project site visits; (3) establish priorities for testing and instruct laboratories to conduct non-routine experiments of special interest; (4) design experimental protocols for screening and detailed drug evaluation, define parameters of response, establish criteria for "activity"; (5) advise Program Analysis Branch (PRA), DR&D, of data retrieval needs and collaborate in establishment of procedures; (6) advise Mammalian Genetics and Animal Production Section, DR&D, of animal requirements; (7) organize, review, and summarize experimental results; (8) instruct laboratories regarding needed additional data; (9) advise natural product chemists under contract to Drug Development Branch of crude materials worthy of fractionation and isolation; (10) conduct studies related to the biological quality control of experimental clinical formulations; (11) conduct studies leading to solution of drug evaluation related problems arising during toxicological study or early clinical trial of new drugs; (12) design and conduct basic research leading to the development of improved assays for screening.

Senior staff serve on the Drug Evaluation Committee (DEC), chaired by the Chief, DEB, which recommends to DCT staff materials which meet criteria for acceptance as clinical candidates. Four of the staff constitute the DEC's Data Review Subcommittee (DRS) which submits to full committee materials meeting minimal criteria for significant and reproducible activity. Staff collectively form the Screening Committee (chaired by the Section Head) which assigns materials that pass Decision Network 2 (DN-2) to contract laboratories for initial route and schedule dependency studies and for additional testing against a limited spectrum of signal rodent tumors; and summarizes data for consideration at the Decision Network 2B and subsequent stages of the linear array. Thus, the Screening Section Staff represent a major part

of an integrated program for the evaluation of more useful antitumor chemotherapy and the intensive study of new agents.

II. Significant Accomplishments

Accomplishments are summarized below in accordance with performance of specific tasks related to (A) determination of antitumor activity of new agents, (B) selection of candidate drugs for development toward clinical trial, (C) detailed evaluation of new and old drugs of clinical interest, and (D) methodology, research, and development.

A. Determination of Antitumor Activity of New Agents (Primary Screening)

Primary screening consists of the original testing of synthetics, plant, fermentation, and animal products submitted to DR&D. Protocols for screening are designed to uncover initially a modest number of materials which are then subjected to progressively exacting requirements designed to select those with the greatest antitumor potential. During calendar year 1972, 15,770 new synthetics, 10,434 new crude plant extractions, 2,712 new crude fermentation products, and 1,669 new crude animal products were submitted to the screening program. All synthetic materials are tested against the L1210 tumor system by the every fourth day (Q4D) and the chronic daily treatment (QD1-9) schedules to the extent permitted by the supply of compound. Crude natural products are tested by the same schedules, however, the mouse leukemia P388 tumor system is utilized for this testing. It should be noted that the supply of compound always permits the testing of crude natural products by both the Q4D and QD 1-9 schedules. Since this represents a large commitment of test capacity, a study was carried out based upon the confirmed active plants for the first year (September 1971 thru August 1972) for which this protocl was in effect in order to ascertain whether or not it actually appeared necessary to use both of these schedules. This analysis indicated that 45.1% of the crude plants completed during this period were negative on the Q4D schedule and active on the QD1-9 schedule, while 23.5% were active on the Q4D schedule and negative QD1-9. The remaining 31.4% were active on both schedules. Since it appeared that a substantial number of confirmed actives would be missed if either schedules were dropped from the routine protocols, we have continued to use both. During this calendar year, the increased test capacity made it possible to expand the evaluation of the B16 tumor systems as a potential slow-growing model. Utilizing the participation of seven contract laboratories and the Screening Section in-house laboratory, 21 compounds were evaluated and the data submitted for statistical analysis. Based upon this work, the protocol has been established for the utilization of this tumor system for compound evaluation. Expanded studies have also been carried out with the Lewis Lung mouse carcinoma (LL) and work was modified and essentially completed with the first generation transplant AKR leukemia. Statistical evaluation of the AKR first generation system indicates a high correlation with the spontaneous system.

In October of 1972 a joint meeting was held with the staff of DR&D and chemists having synthesis contracts, protocols were developed and presented by the screening section and logistics worked out so that all contract analogues now go on test within one week or less after receipt. Rapid testing utilizing the modified L1210 protocol was also conducted as part of the Nucleoside Study. In vitro (KB) screening continued against all new crude plant and animal products and fractionation products from crude plant extractions which had demonstrated correlative in vivo activity. Developmental work was carried out to supply the KB system with L1210 and P388 cells in culture for use in following fractionations of crude plant and animal extractions. Synthetics have also been submitted for KB testing in order to elicit indications of biological activity for selection of candidates for testing in tumor systems under development and in biochemical screens.

B. Selection of Candidate Drugs Toward Clinical Trial

During calendar year 1972, 375 new synthetic materials passed the initial stages of screening. This is a direct reflection of the increased input of materials in the higher test capacity and represents an increase of 180 materials over calendar year 1971. The DRS reviewed 322 materials and the DEC, 127 (Table I). Thirteen were judged to have met current in vivo criteria for acceptance as clinical candidates and were referred to DCT staff for DN2 consideration. A total of 9 drugs passed DN2. Table II lists the materials which passed DN2 in calendar year 1972 and the experimental basis of the selection of each. It should be noted that the low number recommended for DN2 consideration is a reflection of the time-lag necessary to acquire additional compound and complete testing after identification of the material as an initial active and its presentation for consideration. Another factor, of course, is the large number of analogs most of which are not presented because, although active, the activity is inferior to that of the parent already in clinical trial. Examination of the basis for selection for clinical candidates shows that 5 agents met DN2A criteria in L1210. Since the L1210 is a relatively insensitive tumor, this reflects the general experience with this system. The positive correlation between L1210 activity and clinical efficacy indicates that it should continue as our principal primary screen, with P388 to be used for initial screening of crude natural products. Use of these procedures routinely and selective use of B16 and LL would have uncovered almost all drugs with established activity against human solid tumors. Plans are being developed to modify screening procedures to permit a substantial increase in synthetic input with no increase in cost. Possible changes in screening practice are discussed relative to expected yield of DN-2A candidate drugs, correlations with clinical activity, and cost-efficiency in the current Organization Report of the Chief, DEB.

C. Detailed Evaluation of New and Old Drugs of Clinical Interest

The Screening Section is largely responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN2 including route

and schedule dependency tests, combination chemotherapy, studies of comparative effects against fast and slow-growing tumors, and other appropriate studies to determine the extent to which modifications in drug usage might increase therapeutic efficacy leading to cures of experimental tumors. The section reviews and summarizes such information for consideration of Chemotherapy Staff at Decision Points subsequent to initial acceptance. The section maintains the Selected Agents (SAC) File comprising comprehensive records of experimental results of the diverse assays, for active drugs and other agents of special interest.

1. Route and Schedule Dependency Studies

During the calendar year 1972, 45 compounds were subjected to a total of 48 route and schedule dependency studies; 33 against L1210, 5 against P388, 8 against Lewis Lung Carcinoma, and 2 against W256. The Screening Committee reviewed and submitted to the Chief, DEB, summarized reports of such studies for 17 compounds. Initial route and schedule testing is designed to determine the effect on therapeutic effectiveness when the interval between treatment is varied. The usual Type I route and schedule dependency test involves IP treatment on Day 1 only, QD for nine days, every fourth day X 3, every eighth day X 2, every three hours on Day 1 only, every three hours q4d, and every three hours q8d. In addition, attention is given to the determination of activity via the SC route as indication of the effectiveness of parenteral treatment at a site different from that of tumor implantation, and via the oral route as an indication of absorption from GI tract. The IV route is included when feasible. In general route and schedule dependency tests are conducted using parallel groups of tumor-bearing and normal animals as an aid to estimating the degree to which lethal toxicity influences schedule dependency. It should be noted that the number of schedule dependency tests conducted exceeds what one might expect from the number of materials which passed DN2. Usually for each drug, studies subsequent to the initial Type I test are required for confirmation of schedule dependency, determination of schedule dependency via various routes, and determination of schedule dependency of experimental clinical formulations. In addition, the selection of the clinical candidates from active members of a structural class has required comparative schedule dependency testing. Compounds with very extensive activity against the initially observed sensitive tumor may not appear to be schedule dependent because of the extreme sensitivity of the tumor. In such cases, schedule dependency tests are conducted against more advanced forms of the tumor and against other tumors with less inherent sensitivity to the compound.

2. Combination Chemotherapy

A substantial data base has been developed in recent years pointing to the clinical effectiveness of drug combinations, and based upon

this increased emphasis on the conduct of therapeutic trials in animals of new drug combinations which might lead to the development of more effective and curative therapy in the clinic. DR&D has been engaged in combination chemotherapy for a number of years through its contract laboratories, and comprehensive designs for combination therapy studies have been developed. However, program has been somewhat hampered through the lack of a substantial data processing system. Such a system has now been developed and the current combination chemotherapy data processing file now carries the results of 7,423 combination test records; 2,234 of which were added to the file during calendar year 1972. Since this represents a voluminous amount of data an index reflecting the work carried out is circulated routinely to staff members who have indicated an interest. A sample page of this index is shown in Table III. The bulk of combination studies conducted during the past year have been carried out in our intramural laboratory (Project Report, Serial No. NCI-3578), at Microbiological Associates, Inc. (68-3728) and at the Southern Research Institute (71-2098). These laboratories collaborated in a concerted effort to initiate evaluation of all compounds in development toward clinical trial in limited combination studies. Related major findings are summarized in the reports cited above, in the Annual Progress Reports of the contract laboratories involved, and in the current Summary Report of the Chief, DEB.

3. Biological Quality Control of Experimental Clinical Formulations

As a support function, we are collaborating with the DDB, Clinical Drug Distribution Section, in comparing the in vivo activity of formulated drugs with the activity of the bulk compound. The screening system which signaled the activity of the bulk drug is used for this comparison. In addition, considerable effort has been expended in studying the toxicities of non-routine vehicles anticipated for clinical formulation. Such studies can be extensive inasmuch as the potential toxicity of a vehicle must be examined carefully, especially when intensive parenteral treatment schedules are indicated. In the past year, comparisons of experimental and final formulations with bulk material have been carried out for 19 drugs in development toward clinical trial and were reported to the Drug Distribution Section.

4. Further Evaluation of "Deferred" Compounds

Among those compounds maintained in the SAC file are a group which have demonstrated reproducible activity in vivo but lack sufficient activity to be considered as clinical candidates. Since many of these represent new classes of compounds, we re-examine these "deferred" materials to the extent permitted by the amount of material available. These materials are tested against new test systems as the B16 Melanoma and the Lewis Lung Carcinoma, new protocols, and the Type I schedule dependency regimens. One of these materials (NSC-51143) has just recently passed DN-2A. In collaboration with the Southern Research group (Contract NIH-71-2098), 173 agents

which had been deferred on the basis of L1210 activity were re-examined as potential candidates for schedule dependency testing to determine whether the activity required for DN-2A passage might be achieved on a treatment schedule that was not used for primary screening. Of these, 28 were selected for schedule dependency studies on the basis of (1) chemical uniqueness relative to known anti-tumor drugs, (2) potentially interesting mechanisms of action, and (3) reproducibility of existing primary screening data. Ten of these have been tested to date. Four of these ten now meet the DN-2A criteria and will be scheduled for confirmation testing and evaluated further.

5. Other Studies

Additional studies of materials which are in early phase 1 clinical trial or regarded as the most likely candidates for early clinical trial included investigations related to the (a) influence of the tumor site on drug effectiveness (e.g., effects against SC-inoculated tumors or intracranially inoculated tumors as an indication of the hindrance to therapy of the "blood-brain-barrier"), (b) effect of new therapy on the development of resistance or on overcoming resistance to treatment, and (c) mechanisms of drug action.

Special studies are also conducted to meet specific requests addressed to the Screening Section. For example, in an effort to supply information requested by the Baltimore Cancer Research Center of NCI, work is continued at Hazleton Laboratories (72-3704) utilizing the intracerebrally implanted ependymoblastoma to obtain information regarding specific drugs and their schedules against brain tumors. To date, 33 drugs have demonstrated reproducible activity in this tumor system.

D. Methodology (Research and Development)

Research and development consists primarily of studies to establish experimental designs which may lead to improved methods of drug evaluation and to the selection of drugs with greater clinical potential. Current test systems are constantly being evaluated in order to evaluate percentage of active materials being elicited, and to attempt potential modifications in protocol in an effort to reduce the total level of testing. Results of such testing are presented to screening contractors and discussed in the two meetings held with these contractors. Suggestions are also solicited from contractors as to methods for improving the screening program.

With the establishment during calendar year 1972 of a protocol for the utilization of the B16 melanoma tumor and a tentative protocol established for utilization of the Lewis Lung Carcinoma, modest efforts have been set forth in the evaluation of other potential slow-growing solid tumor models (e.g. P815 mastocytoma, currently under study in our in-house laboratory). During the summer of 1972, Dr. Hagemann of Allegheny General Hospital described, during a seminar he gave at the

NCI, a line of P815 mastocytoma which he reported to be readily transplantable from cell culture and exhibited slow growth characteristics in vivo. In our own laboratory it is now in its 25th transplant generation and characterization studies using drugs have begun.

At MAI (73-3728) S-91 Cloudman Melanoma and Carcinoma 1025 were selected by the DEB for further study on the basis of the fact that available data showed that they were sensitive to many of the drugs ranked high for activity against human solid tumors. MAI is characterizing these tumors to see if they would be satisfactory animal models to use for predicting drugs that should be given clinical trial for solid tumors.

Mason Research Institute (71-2259), based upon work done under another contract, has studied the 13762 Mammary Adenocarcinoma in rats, and it appears to be a satisfactory animal model with potential as a representive slow-growing tumor. Drugs reported to be active against human solid tumors are now being tested against this system.

III. Related Projects

September 1972 saw the publication in Cancer Chemotherapy Reports, Part III of the revised screening protocol representing the first complete revision to be published since 1962. Publication of these revised protocols has again made available to the scientific community a comprehensive and detailed source of information on the technical procedures utilized in our massive screening program at the time of publications although screening procedures are continually modified as a consequence of feedback from clinical trials.

In order for the screening program to function properly, all contract laboratories must employ tumor lines with uniform biological characteristics and host animals of uniform high quality. To achieve this uniformity, the Screening Section maintains frozen tumor banks at specified laboratories and from these, new tumor lines are dispensed to all screening laboratories at specified intervals. In addition, the tumor lines are monitored by the Screening Section and if it appears that the characteristics of a tumor line in use may be changing, new tumor lines are requested and sent to all contract laboratories from the tumor bank.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section. Selected screening contract laboratories perform studies related to the diagnosis and control of animal disease and to provide the quality control necessary for maintenance of the quality of the animals utilized in the DR&D program.

The Screening Section, as the major user of the DR&D data processing system, continues to work closely with PRA by advising and assisting in the planning of modifications and the monitoring and review of data necessary to an ongoing data processing system. In addition, we continue to review for need, all requests for special queries to our data files.

IV. Proposed Course of Projects

The Screening Section will continue the supervision of testing and the evaluation of data for synthetic compounds, plant, animal and fermentation products which are submitted to the DR&D. Emphasis will continue to be placed upon several diverse but related projects of interest to the Division of Cancer Treatment Program of NCI. Utilization of in-house laboratory facilities to provide rapid and accurate evaluation of both test systems and compounds of major program interest will continue. This will involve basic research in areas related to cancer chemotherapy and pilot systems aimed at devising new and improved tumor assays prior to their establishment in contract laboratories. Effort will continue both in the intramural program and in the contract areas to devise, implement, and evaluate more sophisticated systems to uncover drugs of greater potential clinical usefulness, especially against human "solid" tumors.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

TABLE I

Status of Active Compounds
Calendar Year, 1972

New Actives	375
No. of compounds reviewed by DRS and DEC	449
DRS	322
DEC	127
Recommended to Assoc. Sci. Dir., DR&D as candidates for Chemotherapy Staff (DN-2) consideration as potential candidates for clinical trial	13
Passed DN-2	9
Route and schedule dependency studies (experiments) scheduled	48
No. of compounds reviewed by Screening Committee	17

TABLE II

COMPOUNDS THAT PASSED DECISION NETWORK TWO DURING 1972

NSC No.	COMPOUND NAME	DATE	BASIS OF ACTION
104801	Acrylic acid, 3-p-anisoyl-3-bromo-, sodium salt	5/15/72	2C-Clinical Activity in Czechoslovakia
139490	Homofolic acid, 5,6,7,8-tetrahydro-5-methyl-	1/10/72	Replaced NSC-89473 - 3LE31 and 3LE31/FR8
141633	Homoharringtonine	2/28/72	Replaced NSC-124147 - P388
142982	Thioxanthen-9-one, 1-[[2-(diethylamino)ethyl]amino]-4-(hydroxymethyl)-, monomethanesulfonate (salt)	2/28/72	3LE21
143057 D	Quinaldine, 4,4'-(hexamethylenediimino)bis[6-amino-, dihydrochloride	8/14/72	3LE21
143414	2,7-(Epoxypentadedeca[1,11,-13]trienimino)naphtho[2,1-b]-furan-1,11(2H)-dione, 8-[N-(4-benzyl-2,6-dimethyl-1-piperazinyl)formimidoyl]-5,-6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20-22-heptamethyl-, 21-acetate, cis-	2/28/72	2C-MLV Induced Leukemia
153858	Maytansine	2/28/72	P388
163062	Triptolide	8/14/72	3LE21
163063	Tripdiolide	8/14/72	3LE21

TABLE III: SAMPLE PAGE OF

DRUG RESEARCH & DEVELOPMENT, DCT, NCI

COMBINATION CHEMOTHERAPY

COMBINATION CROSS-REFERENCE

REPORT DATE 2/22/73

Chemical Name	NSC 1	NSC 2	Screener	Control	Test System
Methotrexate	740	135758	40	0123	3LE21
Thioguanine	752	1895	08	C164	3AA2
			08	C443	3LE21
Nitrogen Mustard	762	740	01	0125	3LL36
Nitrogen Mustard	762	22877	02	4789	3LE21
			02	5649	3LE21
Nitrogen Mustard	762	125066	01	0157	3LL16
			01	0157	3LL36
Nitrogen Mustard	762	402838	40	0134	3LE21
			40	0142	3LE21
			40	0162	3LE21
Cycloleucine	1026	26271	01	0661	3LE32
Cycloleucine	1026	63878	01	2004	3LE32
Guanazole	1895	740	01	1644	3LE31
Guanazole	1895	752	08	C164	3AA2
			08	C443	3LE21
Sodium EDTA	2760	82151	01	0254	3AA3
			01	0257	3AA3
			01	0261	3AA31
			01	2003	3LE31
			01	2033	3LE31

Serial No. NCI-3578
1. DCT, DR&D
2. Drug Evaluation Branch
3. Screening Section
4. Building 37

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Basic Studies for Drug Evaluation Utilizing Antitumor Screening

Previous Serial Number: Same

Principal Investigator: B. J. Abbott

Co-Principal Investigator: D. Abraham, M. Baratta, R. Geran, E. Wagshal

Cooperating Units: Drug Development Branch (R. Engle), (P. Davignon); Southern Research Institute (Contract 71-2098, H. Lloyd and W. R. Laster); Illinois Institute of Technology Research Institute (Contract 68-1021, A. Shefner).

Man Years: Total : 4

Professional: 1 1/4

Other : 2 3/4 (includes only 1 full-time person, other staffing with part-time employees)

Project Description and Objectives

The Screening Section intramural laboratory conducts basic studies related to in vivo antitumor screening emphasizing the development of new and improved methods, and the detailed evaluation of drugs of interest to the Division of Cancer Treatment Program.

Methods Employed

Studies were conducted mainly in mice using transplantable leukemias L1210 and P388, melanoma B16, and Lewis Lung carcinoma; and in first generation transplanted AKR Leukemia, and the P-815 Mastocytoma. Established methods and new procedures developed within this project were used.

Major Findings

I. Studies related to the design of protocols for screening.

A. The Protocol Committee completed and published the 3rd edition of "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems" (see Geran et. al., Cancer Chemotherapy Reports, Part 3, Vol. 3, 1972). Although screening contractors are informed of protocol changes via specific instructions from staff, the many changes in procedure and experimental design since publication of the "Protocols" in 1962 had not been presented in comprehensive form to the scientific community until this past year. A major objective of the Screening Section's intramural laboratory program is to conduct experiments to provide information necessary for the design of rigid screening protocols used in all contract laboratories.

B. Comprehensive studies (with W. R. Laster, Jr.) of the effects of the presence of food in the stomach on the activity of orally administered drugs led to the conclusion that increased or decreased absorption was not a fixed consequence of stomach contents but depended on the drug itself. Also, it was found that, although fasted mice exhibited a diminished amount of food in the stomach, it was not clear that fasting resulted in a smaller quantity in the small intestine. When route dependency studies of a drug in development are conducted, little is known of either the potential site of absorption from the GI tract or the binding characteristics of the drug itself. The Protocol Committee concluded that neither feeding nor fasting was a completely tenable procedure under all circumstances. Thus, the requirement to fast animals receiving oral drug was deleted from the 1972 edition of screening protocols. Studies of oral activity are of critical importance to decisions relative to the further development of an active drug, affecting primarily decisions relative to formulation development. For an orally active drug, the ratio of oral to parenteral therapeutic dose is also important. Therefore, future route dependency studies will include both "fasted" and fed groups.

C. It was found that the number of untreated leukemic (L1210) mice caged together (from one to twenty-one) did not significantly affect their survival time. New screening procedures under consideration (see current Organization Report, O.C., DEB) involve the use of as little as 3 and as many as 10 mice per treatment group depending on the status of the material. Thus, it was considered important to have information on the influence of population density on survival.

D. Studies of the influence of the vehicle for injection on drug effectiveness and animal survival were continued. These included the toxicity to mice of commonly used

vehicles given by various treatment regimens as well as the effects of pH on the toxicity and anti-tumor activity of vehicles or drug preparations (with P. Davignon).

E. MTX (NSC-740), 8-Azaguanine (NSC-749), and BCNU (NSC-409962) retained their characteristic anti-L1210 activities whether given IP as solutions, saline suspensions, or CMC suspensions. However, it is known that the physical state of an injection preparation can markedly affect its treatment schedule dependency.

II. B16 Melanoma and Lewis Lung (LL) Carcinoma

Studies were continued to develop a data base relating to the use of B16 and LL as "solid tumor" models. Using compounds of known clinical utility, these experiments are designed to evaluate the reproducibility of these systems.

III. L1210 and P388 Leukemias

In order to provide rapid feedback of information to the Drug Evaluation Committee and other program units, 46 experiments in L1210 and 20 experiments in P388 were carried out. These included comparison studies of parent compounds with new analogs and evaluation of samples of bulk drugs prepared for clinical trial (with R. Engle), evaluation of clinical formulations (with P. Davignon), and rapid evaluation of new structures of special interest. A limited number of combination chemotherapy studies were conducted at the specific request of DR&D staff members.

IV. P815 Mastocytoma

Work continued to evaluate characteristics of the P815 Mastocytoma which has been reported to exhibit an unusually long doubling time when implanted SC. This tumor line was established in vivo and, after continuous transplant, showed only insignificant changes in growth characteristics. Exploratory drug tests against P815 have been initiated.

V. First Transplant Generation of AKR Leukemia (with A. Shefner and H. Lloyd)

Statistical evaluation had indicated a good correlation between responses to drugs of the first transplant generation of AKR and the spontaneous AKR lymphoma. Thus, in collaboration with IITRI (68-1021) and the Southern Research Institute (71-2098), we are making a concerted effort to complete testing against the first generation transplantable AKR tumor with all drugs that have been tested against the spontaneous tumor. Four AKR experiments were conducted with these drugs in this laboratory.

Final evaluations are being made of this system and publication is in preparation.

Proposed Course

Continuation of studies related to the development of improved assays of drug evaluation, structure-activity studies of combination chemotherapy, and studies devoted to the solution of special problems arising during formulation development, toxicological examination, and clinical trial of active antitumor drugs is anticipated.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

Serial No. NCI-3583
1. Division of Cancer Treatment
   DR&D
2. Drug Evaluation Branch
3. Office of the Chief
4. Building 37

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Modification of Division Patterns and Immunogenicity of Neoplastic Cells by Interaction of their Surfaces with Microenvironmental Macromolecules

Previous Serial Number: Same

Principal Investigator: Muriel Lippman

Co-Principal Investigator: None

Cooperating Units: Microbiological Associates, Inc. (I. Kline), Battelle Memorial Institute (R. Folk and A. Barker)

Man Years: Total : 2
Professional: 1
Other : 1

Project Description:

Objectives:

1. The project aims at exploiting those changes in cell surface which accompany neoplastic transformation with the object of finding cell division inhibitors which selectively bind to transformed cells. To this end polysaccharides, glycoproteins, proteins, and synthetic polymers are to be obtained and tested for surface binding and cell division inhibition activity on a variety of cell types in culture.

2. In the case of manifest cancer the immune responses evoked by the malignant cells have failed to effect a complete rejection of the neoplastic cells. Evidence exists that attributes this failure to the weak nature of the cancer cell as an immunogen and to the phenomenon of enhancement frequently observed with sarcomas and carcinoma but not with leukemias. Studies are carried out with the aim of increasing the immunogenicity of the neoplastic cells and preventing some aspects of enhancement.

3. Binding of a particular polymer to a cell surface depends on cell type but also on physiological age of the cell with respect to the cell and culture cycles. Conventional as well as new methods of cell separation are developed in order to investigate adsorption as a function of the cyclic behavior of the cells.

4. New methods are to be developed for rapid, microscale, in vitro tests suitable for screening small quantities of compounds which may provide more information than measure of the cytotoxic action of the agent. Of special interest are microculture methods for human tumor cell lines.

Methods Employed:

Along with standard methods a substantial part of the project efforts are devoted to the further development of unique methods of cell separation, analysis, and culture which are needed to realize the aims and objectives of the project.

Major Findings:

Studies with the TA3 Tumor System

The TA3 Tumor, obtained last year from Dr. T. Hauschka at Roswell Park, originated as a mammary adenocarcinoma in the A strain mouse in 1949 and was subsequently transplanted and converted to the ascites form. Two variants of this subline are available. The TA3K1 subline which retains its specificity for mice bearing H-2^a histocompatibility antigens and the TA3Ha subline which transgresses histocompatibility barriers and grows with varying degrees of virulence in different strains of mice. Three different kinds of studies employing this system were carried out during the year.

I. Development of an assay system for determination of modifications in tumor cell immunogenicity induced by drugs.

II. Development of an assay system for determination of the immunosuppressive (IS) or immunoaugmentative (IA) activities of selected drugs.

III. In vitro studies on the binding of various macromolecules to the surfaces of these variant sublines.

Significant results in these studies are summarized below:

I.a. This study initially involved injection of the two tumor sublines into a variety of hybrid mice in order to provide data concerning the takes and progressive lethal growth of these sublines in animals of differing histoincompatibilities. Such data is a necessary prerequisite for determination of how surface bound macromolecules modify immunogenicity.

The availability of both the syngeneic A strain and allogeneic BDF_1 hybrids made the latter strain the hybrid of choice for the

earliest studies. Accordingly data on takes and survival times for the sublines were obtained in normal and immunosuppressed animals. As a result of these experiments BDF_1 mice which had received and, as expected, rejected TA3K1 tumors were available for further study. These mice were subsequently challenged with TA3Ha cells at various inoculum levels. The TA3Ha cells normally take and produce lethal tumors in 100% of BDF_1 mice with survival times dependent upon the number of cells inoculated. Injection of TA3Ha cells into BDF_1 mice previously inoculated with TA3K1 cells produced three types of results all significantly different from controls. These results were as follows:

1. Mice receiving 10^6 or 10^2 TA3K1 cells showed a very high incidence of immunity to the subsequent challenge with 10^4, 10^5, or 10^6 TA3Ha cells. Some individuals which received 10^2 TA3K1 cells were not rendered immune and fell into the next category.

2. Animals which had received 10^3, 10^4, and 10^5 TA3K1 cells had a low incidence of immunity but a high frequency of death with tumor. The survival times of the animals that died with tumor were significantly shorter than those of the controls suggesting enhancement. Experiments are now in progress to determine whether blocking factors are indeed involved.

3. In one experimental design the animals uniformily died with tumor but with survival times almost 100% longer than controls.

These results rule out the interpretation that TA3Ha cells are resistant to immune cells and suggest weak immunogenicity as the explanation for their ability to grow in allogeneic hosts. They also show that factors which determine immunity, partial protection or enhancement are a function of quantitative variation in immunizing inoculum level, challenge level, and the immune status of individual host animal. The results of these studies are detailed in "Immunity to a TA3 Tumor Subline which Grows in Allogeneic Hosts Elicited by Strain Specific TA3 Tumor Cells", Cancer Research, April 1973.

These results have an important bearing upon problems involved in application to immunotherapy. Specifically the effectiveness of the immunotherapy may be a function of the tumor burden of the individual and vary in effectiveness depending upon prior drug, radiation or surgical treatment designed to reduce the tumor burden as well as the effect of these treatments on the immune status of the host.

b. Study of the TA3Ha and TA3K1 tumors in hybrid strains other than BDF_1 has been more difficult due to problems in breeding sufficient numbers of F_1 hybrids of the histocompatibilities selected. TA3K1 cells are uniformily rejected in all hybrids while TA3Ha cells take and grow in all hybrids tested. An hierarchy of barrier

strength was evident from the cell inoculum levels required to give 100% takes in the different strain as shown below. These data are useful in measuring modification of immunogenicity as a function of antigeneic specificities.

Growth of TA3 Tumor in Various Hybrids

Strain	Lowest inoculum level resulting in 100% Takes	Mean survival time at that level (days)
BDF_1	10^1 - 10^2	24.1
DBA/2 x P/s and R	10^4	21.3
DBA/2 x C/St and R	10^5	17.3
C3H x C/St	10^6	14.8

II. The TA3 system appeared to have characteristics which make it uniquely suited as an assay system for measure of the immunosuppressive (IS) or immunoaugmentative (IA) action of particular drugs.

In order to determine whether the system could be used in this way preliminary studies with the TA3K1 cells in BDF_1 mice were carried out in collaboration with R. Folk and A. Barker at Battelle Memorial Institute.

In these experiments a single injection of test drugs was given 24 hrs. prior to injection of TA3K1 tumor into BDF_1 allogeneic mice. Immunosuppression is reflected in growth of tumor in these hosts which normally completely reject TA3K1.

Different drugs dosages were used at three different tumor cell inoculum levels. The degree of immunosuppression produced by the test drugs at the highest completely non-toxic dose used in the experiment, is expressed as the percentage of animals receiving that drug which succumbed to tumor by 30 days. The immunosuppressive activity was then rated. If 90-100% of the animals succumbed to tumor the IS activity was maximal and rated +4, 70-89% deaths were rated +3, etc. The following table lists the results of this study.

Percentage BDF_1 mice which died with tumor
following drug treatment and i.p. injection of TA3K1 cells

	Highest non toxic dose used (mg/kg)*	10^6 TA3K1			10^4 TA3K1			10^2 TA3K1		
		Treat.	CONT.	IS	Treat.	CONT.	IS	Treat.	CONT.	IS
CYTOXAN (NSC-26271)	150	100	0	+4	50	0	+2	80	0	+3
BCNU (NSC-409962)	25	100	0	+4	90	0	+3	80	0	+3
CCNU (NSC-79037)	60	90	0	+4	40	0	+1	0	0	0
MeCCNU (NSC-95441)	25	60	0	+2	70	0	+3	50	0	+2

* Other doses tested were: Cytoxan 200, 100 mg/kg. CCNU 30, 15 mg/kg.
BCNU 50, 12.5 mg/kg. MeCCNU 50, 12.5 mg/kg.

IS = Immunosuppressive rating 90 - 100% = +4 70 - 89% = +3 50 - 69% = +2
20 - 49% = +1 0 - 19% = 0

Overall IS rating: Cytoxan = +9, BCNU = +11, CCNU = +5, MeCCNU = +7.

Rated in this way the relative activity for all inoculum levels is BCNU>CYTOXAN>MeCCNU>CCNU. However, the immunosuppressive effectiveness is also a function of the level of tumor challenge.

At high levels (10^6 TA3K1 cells):

(BCNU = CCNU = Cytoxan) > MeCCNU

At intermediate levels (10^4 TA3K1 cells):

(BCNU > MeCCNU > Cytoxan > CCNU)

At low levels (10^4 TA3K1 cells):

(BCNU = Cytoxan) > MeCCNU > CCNU

At all levels BCNU has the greatest immunosuppression action at the dose level used. MeCCNU was used at 25 mg/kg since the next highest level used, 50 mg/kg was toxic. It appears some dose between 25 and 50 mg/kg might be optimal for testing of MeCCNU. Similarly the 60 mg/kg dose of CCNU might have been raised slightly to give optimal activity.

In addition to data on the number of animals which succumbed to tumor the study provided information on survival time and incidence of tumor development with subsequent regression. These latter figures are presently being analyzed. Experiments are also in progress which are designed to distinguish between humoral and cell mediated components of the immunosuppression.

III. No difficulty was experienced in culturing the TA3Ha x TA3K1 sublines in suspension in the same medium as is used for L1210 cells. The doubling time is about 24 hrs. for TA3Ha and slightly longer for TA3K1. The two sublines can be readily distinguished by their volume spectra, the TA3Ha being considerably larger. The lines are being used along with L1210 and LM cells to compare binding action of various macromolecules (described in the next section).

STUDIES WITH SURFACE BINDING MOLECULES.

The rationale for examining surface binding macromolecules is to find such macromolecules as will show a) measurable differential binding to malignant cells as compared with normal cells b) to find agents that show selective binding to some tumor cell types as opposed to others c) to find such binding agents as will restore normal patterns of division d) to find surface binders which bind to the malignant cell surface and increase its immunogenicity.

For these studies it is convenient to use cells which grow in suspension so that assay for surface binding does not require trypsinization. It is also valuable to have cell lines with known histocompatibilities and which are well studied in this regard in vivo. The L1210, TA3Ha and TA3K1 are particularly suited for use on all of these counts. The LM cell line is used as a non-malignant control.

a) A polyanion preparation which had previously been shown to bind to LM cells with subsequent inhibition of division was not available in adequate quantity to test in the L1210 system. Another similar preparation was ineffective against the L1210 cells indicating selective binding to non-malignant cells.

b) A variety of preparations which have been reported to bind to malignant cell surfaces have been examined. Among these one preparation of macromomycin a product first prepared by H. Chimura was found to completely block cell division of L1210 and TA3Ha cells. The effective inhibitory concentrations did not cause agglutination of the cells but increased the population of cells of the size equivalent to metaphase or doublet cells. Division did not resume after wash and reculture of the cells. The data on inhibition of division is given in the following tables. Further studies with this molecule are in progress.

The Effect of Macromomycin on Suspension Cultured TA3Ha Cells

Time	Concentration Macromomycin (μg/ml)				
	0	1	2	4	8
(Hrs.)	Count x 10^5/ml (viability % by Trypan Blue Exclusion)				
0	2.0 (100)	2.0 (100)	2.0 (100)	2.0 (100)	2.0 (100)
3	2.0 (100)	2.0 (93)	2.0 (92)	2.0 (92)	2.0 (94)
18	3.8 (100)	2.8	2.1 (97)	2.0	2.0 (100)
26	4.8	2.9	2.0	2.3	2.1
48	7.2	3.7	2.1	2.0	2.0

Effect of Macromomycin on Suspension Cultured L1210 Cells

Time	Concentration Macromomycin (μg/ml) 0	1	2	4	8
(Hrs.)	Count x 10^5/ml (viability % by Trypan Blue Exclusion)				
0	2.1 (100)	2.1 (100)	2.1 (100)	2.1 (100)	2.1 (100)
2	2.1 (100)	2.0 (95)	2.1 (75)	2.1 (90)	2.0 (72)
3	(100)	(96)	(88)	(88)	(83)
19	3.4 (100)	2.3 (90)	2.2 (70)	2.2 (72)	1.9 (70)
27	5.7 (100)	2.4 (93)	2.3 (64)	2.0 (76)	1.9 (74)
44	11.3 (97)	2.4 (91)	2.1	1.9	1.8
	(Cells washed diluted and resuspended)				
0	2.3	1.0	1.1	-	2.1
24	5.8	1.0	1.1		1.5
48	15.9	1.1	1.4		1.5

Significance for the Program:

1. Focus on the cell surface as target for chemotherapeutic agents extends the search for active materials to include compounds which:

   a. have good possibilities for achieving selectivity in cell division blocking between normal and neoplastic cells.

   b. can be used to increase the immunogenicity of neoplastic cells and therefore, may be used in combined chemo-immunotherapy. Such an approach insures a more complete attack on the malignant population than is afforded by chemotherapy alone.

   c. are involved in the normal regulation of cell division in vivo. This approach recognizes that manifest malignancy is a function not only of a transformed cell population but of a permissive microenvironment of the cell.

2. Development of special methods for obtaining unique subpopulation of a given cell type would provide a good tool in the search for agents which would be expected to affect cells in prolonged G_1 or other chemotherapeuically recalcitrant subpopulations.

3. Good in vitro methods on a micro scale which permit testing of agents in small quantity on a variety of malignant cell lines would provide a useful adjunct to the general KB cell test and enable a search for molecules which may affect one specific cell type and not others. The same endeavor in in vivo systems would be prohibitive in terms of the number of animals required, length of time required to get the data and quantity of test agent available. The problem in finding in vitro assays which correlate well with in vivo activity is especially acute where only modest amounts of drug are available and a variety of neoplastic cell types should be studied. Such in vitro systems enable one to sort out direct effects on cell division from immunological and pharmacological problems involved in obtaining effective agents. While agents that are effective when tried in vivo directly are good candidates for further analysis and study, it is also very likely that compounds which do not work in vivo fail to do so for multiple reasons which could be overcome if attacked individually. Such agents are now eliminated from further study and could include many potentially active materials.

Proposed Course:

The proposed course consists in development along the lines described under project description and objectives.

Publications:

See current Organization Report, Office of the Chief, Drug Evaluation Branch.

ANNUAL REPORT

PROGRAM ANALYSIS BRANCH

DRUG RESEARCH AND DEVELOPMENT, DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

PRA provides most of the data with which program decisions are made within DR&D. In many areas, notably drug costs, publications, and literature activity (including Decision Network meetings), PRA supports the entire Division of Cancer Treatment. Major functions include the capture, maintenance, analysis, and dissemination of technical data and information. The Branch maintains the DR&D chemical, biological, and drug costs data banks, and provides information for program-monitoring and decision-making. Other activities include systems management and the publication of program and program-related information.

The doubling of the input to the primary antitumor screening program and the marked increase in testing (including expanded secondary evaluation) during the past year has had a tremendous impact on the entire Branch. Priority handling of contract-synthesized compounds was an added complication but apparently has the desired effect of speeding data to synthesis contractors and thus aiding our targeted research.

In conjunction with our contractors, PRA has recently developed accurate figures reflecting the cost of processing data on materials coming into the screen. These unit costs will enable us to project more readily costs for any level of compound acquisition or testing agreed upon by the DCT staff. The automatic identification of new or duplicate material previously received costs, for example, only about 10% of what would be incurred for retesting a compound previously received without such identification.

PRA has continued to produce reports showing the costs of commercial drugs provided to Cooperative Groups, independent investigators, contractors, and intramural staff. When adriamycin and bleomycin were shown to be very active, PRA developed reports showing the rate of usage and the projected costs to the program for FY 73 and FY 74. The drug costs files are becoming increasingly useful throughout NCI as management tools and among other uses provide a ready list of investigators who must be notified when new information becomes available about a drug in clinical trial. During CY 72, records were kept of the more than $1,315,096 worth of commercial drugs, and $244,288 worth of investigational drugs that were sent to investigators.

Just as PRA supports DDB and DEB, the Sections of PRA are highly interdependent and must work cooperatively in carrying out various tasks, especially in the areas where special analyses of files are required. For example, the Director, DCT, expressed a desire to see the molecular weight distribution of the compounds having special interest to the program (the so-called SAC list). PRA

produced tables and histograms detailing the frequency distribution of the SAC compounds by molecular weight. Of the 3284 compounds, 86 had unknown molecular formulas or were polymers whose exact molecular weight can not be calculated at the present time. As expected, the majority of the compounds had molecular weights between 60 and 840. The highest molecular weight was 3708.4. Clinically active compounds are identified on this chart.

To permit DR&D staff to have easy access to their own data, Information Storage and Retrieval System (ISRS), an interactive data base management and inquiry system, was developed by Dr. Alan Greenfield. It was designed to serve as a quick means of storing and retrieving small special-purpose collections of data. Because of the general need among scientific investigators for such a system, PRA released ISRS to DCRT, who offered it to the entire NIH community in November of 1972. It has also been adopted by others outside NIH.

Project Stride is an important part of the HEW Upward Mobility Package. It is a program which combines on-the-job training with college courses leading to a bachelor's degree in order to move talented, non-professional employees into professional jobs. The Branch supported this program by hiring two Stride trainees in August of this year. One trainee works in the Branch 20 hours a week, as he learns basic programming skills under the direction of a computer programmer. Toward the end of the three-year program, the trainee will be able to function as a productive member of the PRA staff. The other trainee acts as a technical publications editor in the Publications Section. This trainee has become a valuable member of the staff, providing part-time services to the Section while learning skills related to her studies.

Under the Assistant Chief, the biological data processing system has developed during the past year into a more useful data bank than ever before. Not only does it report on and store the data submitted by screening laboratories, but it has become an important source of data for statistical studies in support of the constant evaluation of the primary and secondary screens. As a result, a concentrated effort is now underway to put all of the data on smaller systems tested, but never stored, into computer compatible format so that future studies may include consideration of these experiments (examples are AKR, B16, and C3H).

It is important to note that the biological data processing system not only processes and disseminates screening data to staff, contractors, and suppliers, but in many instances is the only ready source of information needed for program management. Reports produced regularly or on demand aid DDB, DEB, and to a lesser extent PRA in managing their contracts. The data banks are continuously being used to identify compounds in order to schedule testing to determine activity in special systems, and to obtain statistics on the number of materials and the type of testing carried out. In addition, the reports produced from this system are used as sources of data for various DR&D Branch reports.

During CY 72, 30,585* synthetic and natural products were processed. During CY 72, 303,105 in vivo primary screening tests were processed. Of these, 103,681 were carried out against mouse leukemia L1210; 180,027 against mouse leukemia P388; and 19,397 against other transplanted rodent tumors. All materials were tested in cell culture; 25,966 such tests were processed.

Improvements by the Value Engineering Company (the new data processing contractor) coupled with lowered rates at Division of Computer Research and Technology (DCRT) have actually reduced the cost of processing one line of primary test data by about 20%. To encourage cost consciousness among our users, each report prepared in answer to a request of any system automatically includes the computer cost of making the file query.

A major change during the year was that of running the biological data processing system every other week instead of weekly. Although this increased the time lapse between screener submittal of data and the receipt of output reports, there were some definite advantages. The cost of computer processing, postage, mailing, and forms were significantly decreased. The integrity of the file increased with fewer computer runs, and overall accuracy and efficiency were improved because of smoother work flow. Just as importantly, the filing time required of Scientific Records Section was also decreased by about 15%.

Under a contract with Arthur D. Little, Inc., recommendations for expanding and increasing the flexibility of the biological data processing system were prepared. At the suggestion of the Advisory Committee on Synthesis, analysis and programming was provided to place English language messages pertaining to compound evaluation on the Screening Data Summaries. In addition, an investigation was made of methods for providing suppliers with periodic summary reports which would include the supplier's laboratory number, systematic chemical names, and an evaluation of screening data.

The Data Processing and Computer Systems Development Section is primarily concerned with the design, implementation, and installation of the DR&D extended chemical information system for handling chemical structure information. The new system will control and speed the processing of synthetic compounds, as well as maintain inventory control, furnish management reports, provide easier inquiry, and eliminate many manual procedures and files. In designing the system, user needs were reviewed against cost considerations and impending technological advances; decisions were made to phase in desirable system features only when costs would be acceptable. Consultants from government, industry, and universities advised the DR&D staff as critical design options were developed.

The Section demonstrated the feasibility of using computer tape services and data bases from commercial organizations to identify analogs of compounds of

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* All references to materials submitted in this report were taken from the PRA report of Jan. 23, 1973 -- "Materials Added to the Master Files."

interest either in industrial collections or as reported in the literature. The Institute for Scientific Information, Shell Development Co., and Eastman Kodak were particularly useful.

An information storage and retrieval system is operated by the staff of the Literature Research Section; this Section collects and publishes literature on cancer chemotherapy and prepares data summaries, special reports, literature searches, and annotated bibliographies specific to the needs of cancer chemotherapy investigators. In addition, the Section maintains on contract the current-awareness serial Cancer Chemotherapy Abstracts (CCA) and operates the DCT library.

The Literature Research Section filled over 200 requests for references and materials during the year. Information from the published literature and the NCI Contract Progress Reports was provided in the form of abstracts, bibliographies, computer printouts, and copies of articles. Selection of literature for use in Decision Network Review, for meeting FDA requirements for IND filing, and for the evaluation of clinical studies continued as the Section's primary responsibilities. Other responsibilities included the preparation of agent folders for the Decision Network meetings and quarterly reports of the DCT Program Statistics. Table I compares the total program statistics for 1972 with those from 1971. These data were compiled from the Section's quarterly reports.

The Section's work was greatly aided by the computer services provided by the National Library of Medicine (NLM) and the DCRT. Evaluation of other available automated selective dissemination of information services continues in conjunction with the Data Processing Section.

A Section member coordinates NCI's access to NLM's on-line automated bibliographic search system, MEDLINE. In this role, the Section serves all areas of the Institute, providing assistance in the processing of search requests and instruction in the use of the terminal.

In monitoring the contract for Cancer Chemotherapy Abstracts, every effort has been made this year to bring CCA up-to-date and to improve its appearance and organization. Consideration is being given to expanding its coverage to include surgery, radiotherapy, and immunotherapy of cancer.

The Publications Section produces Cancer Chemotherapy Reports (CCR), a primary-source journal containing reports of clinical and experimental research on chemical agents developed in the Cancer Treatment Program by contractors, collaborating institutions, and other investigators. The Section also publishes supplements to CCR on screening data and program information, assists the Cancer Treatment staff with special publications, publishes the Chemotherapy Fact Sheet on drugs, maintains the mailing lists for its own publications and CCA, and clears contractors' manuscripts for publication.

In its 15th year of continuous publication, Cancer Chemotherapy Reports has added several new features and is investigating new ways to more rapidly present information. During CY 72 the Publications Section produced 6 issues of Part 1 (814 pp), 1 issue of Part 2 (353 pp), and 2 issues of Part 3 (181 pp). In FY 73 CCR began a monthly publication schedule, devoting several issues of

Part 3 to the Proceedings of meetings (such as the annual contractors conference and the International Workshop on Lung Cancer) and screening and toxicology protocols. Although CCR is continuing to publish 6 bimonthly issues of Part 1 (containing current research), an increasing emphasis will be placed on program information. Over the past four years the number of pages published has increased dramatically (40%).

A total of 166 contractors' manuscripts were cleared for publication in 1972.

The role of the Scientific Records Section (SRS) has been changing to provide more analytic as opposed to purely supportive services. In this regard, SRS performed a number of file analyses during the year including that of special-interest compound acquisition by year. The characteristics of the master and special-interest files were compared in preliminary work to determine methods of assigning priorities for testing new synthetic compounds. SRS supported other parts of DR&D in providing data for structure-activity relationship studies; for flexibility in manipulation, these data were stored in machine-readable form.

SRS plays an important role in all phases of the processing of chemicals tested against cancer, from the first drawing of a structure to the retrospective correlation of chemical structure with biological data. Section personnel direct the initial chemical-structure file building, participate in the shipping of compounds to screeners, maintain master card and microfilm files, and monitor certain inputs and outputs of the biological data files.

Because of the large size of the chemical and biological master files and their accelerated growth rate in the past year, the Section has increasingly turned to automated methods for the building and maintenance of its files as well as for the retrieval and analysis of the data they contain. As a step toward better handling of the increasing volume of information and the need for more sophisticated analysis, the Section cooperated in monitoring the University of Pennsylvania contract to develop a new chemical information system. This cooperation will continue and expand as new processing methods have an impact on SRS.

BRANCH STAFF

PRA is composed of a staff of 35 persons consisting of 3 professional senior staff members (GS-13 and above), 10 other professional staff members (GS-09 and above), 18 technical clerical staff members, 2 Stay-In-School people and 2 Project Stride trainees.

Chief, Program Analysis Branch	Barbara R. Murray
Assistant Chief	Rhoda R. Mancher
Head, Data Processing and Computer Systems Development Section	Sidney Richman
Act'g Head, Scientific Records Section	George F. Hazard, Jr.
Head, Literature Research Section	Dorothy Algire
Head, Publications Section	Pamela Hutchins

Staff have primary responsibility for 7 contracts. PRA-monitored contract activities were funded at approximately $1,395,000 as of January 31, 1973. The relationship of PRA-monitored contracts to activities outlined in the DCT Linear, Supplementary, and Exploratory Arrays is shown in Tables II through IV.

HONORS AND AWARDS

1. Barbara R. Murray, Nominated by DHEW for the Horace Hart Award for Distinguished Public Service in the Field of Printing and Publishing. Presented by the Education Council of the Graphic Arts Industry, Inc., December 27, 1972.

2. Barbara R. Murray served as Chairman of the COSATI Task Group on Chemical Information Systems.

3. Miss Lynn R. Reutlinger, Summer Aid, Program Analysis Branch, received a cash award for outstanding performance.

PUBLICATIONS AND PRESENTATIONS

1. Mancher, R. R.: Mechanism of flow of new agents and data through the primary screen. Presented at the Third Joint Working Conference, Hunt Valley Inn, Hunt Valley, Maryland, October 2-4, 1972.

2. Mancher, R. R.: Interpretation of raw data sheets. Presented by the Synthetic Contractors Workshop on Compound Processing and Screening, National Cancer Institute, October 26, 1972.

3. Greenfield, A. J. and Maloney, C. H.: Using Partitioned Data Sets as Back-up Storage for CPS. Interface 36:29-31, 1972.

4. Kalikow, A. K., Sloane, E. M., and Richman, S.: Machine Searching of Natural Language Text vs Manual Searching of Printed Indexes. Abstr Papers Amer Chem Soc, 164 Meet. CHLT 009, 1972.

5. Richman, S., Hazard, G. F., Jr., and Quinn, F. J.: Mechanized Methods for Compound Acquisition. Abstr Papers Amer Chem Soc, 164 Meet. CHLT 004, 1972.

6. Milne, M., Plotkin, M., Hill, H. N., Richman, S., Hazard, G. F., Jr., and Murray, B. R.: A Systematic Analysis of Substructure Search Questions for the Chemotherapy Program, NCI. Abstr Papers Amer Chem Soc, 164 Meet. CHLT 001, 1972.

7. Milne, M., Plotkin, M., Powers, R. V., Hill, H. N., Lefkovitz, D., and Hazard, G. F., Jr.: Design of Screens and Indexes for an On-Line Substructure Search System. Abstr Papers Amer Chem Soc, 164 Meet. CHLT 003, 1972.

8. Greenfield, A. J. and Maloney, C. H.: Introducing ISRS to Users. Interface 37:1,8-9, 1972.

9. Shamos, M. I.: Molecular Weight Calculations Made Easy. Interface 35:1,4,5, 1972.

Table I

Division of Cancer Treatment Program Statistics -- January-December 1972

	1972	1971	
1. Materials Added to the Master Files*			
Synthetic Compounds	14,781	9,066	
Fermentation Products	2,712	1,215	
Plants	10,434	2,825	
Animal Products	1,669	1,120	
Total	29,596		14,226
2. Tests Performed			
L1210 lymphoid leukemia	103,681	102,424	
P388 lymphocytic leukemia	180,027	53,449	
Walker carcinosarcoma	2,770	4,368	
B16 melanoma	6,028	1,423	
Lewis lung carcinoma	4,141	2,803	
		700	AK leukemia
Other tumors	6,458	1,080	
Total in vivo (excluding toxicity tests)	303,105		166,247
Toxicity	2,900	2,835	
Cell Culture	25,996	12,485	
Chemical Analysis	231	392	
Total	332,232		181,959

* These figures are derived from the Drug Research and Development Biological Data Processing System and differ from those generated by the Drug Development Branch, which logs in materials when NSC numbers are assigned. A material first enters the Data Processing System when its receipt is acknowledged by the screener which may be 2 to 4 months later.

3. Materials having Confirmed Activity in Primary Screen

	1972	1971
Synthetic Compounds	375	
Crude Antibiotics	204	
Crude Plant Extracts	189	
Crude Animal Extracts	54	
Total	822	377

4. Compounds Passed Decision Network IIA

NSC NO.	NAME	SYSTEM	DATE
104,801	Cytembena	Clinical	5/15/72
139,490	Homofolic acid, 5,6,7,8-tetrahydro-5-methyl-	L1210	1/10/72
141,633	Homoharringtonine	P388	2/28/72
142,982	Hycanthone monomethanesulfonate	L1210	2/28/72
143,057	Quinaldine, 4,4'-(hexamethylenediimino)bis[6-amino-, dihydrochloride	L1210	8/14/72
143,414	Rifamycin, 4-benzyl-2,6-dimethyl	MLV induced leukemia	2/28/72
153,858	Maytansine	P388	2/28/72
163,062	Triptolide	L1210	8/14/72
163,063	Tripdiolide	L1210	8/14/72

	1972	1971
Total DN-2A	9	18

5. Compounds Passed Decision Network IIB

NSC NO.	NAME	DATE
4,728	1,3,4-Thiadiazole, 2-amino-	1/10/72
71,795	Ellipticine	8/14/72
126,771	1,4-Naphthoquinone, 2-(3,3-dichloroallyl)-3-hydroxy	8/14/72
132,313	Galactitol, 1,2:5,6-dianhydro-	8/14/72
141,537	Anguidine	5/15/72
145,668	O2,2'-Cyclocytidine, monohydrochloride	8/14/72

	1972	1971
Total DN-2B	6	9

6. Compounds Passed Decision Network III

77,037	d-Tetrandrine	5/15/72
95,441	Methyl-CCNU (parenteral)	5/15/72
121,210	DHEA Mustard	2/28/72
404,241	Adenine, 9- -D-arabinofuranosyl-	1/10/72

Total DN-3	4	7

7. Compounds Passed Decision Network IV

None

8. Compounds Passed Decision Network V

None	0	1

9. IND's filed

58,514	Chromomycin A_3	7/13/72
68,075	Thalicarpine	8/24/72
122,819	Epipodophyllotoxin, 4'-demethyl-, 9-(4,6-O-2-thenylidene-β-D-glucopyranoside)	9/15/72

NSC NO.	NAME	DATE	1972	1971
141,540	Epipodophyllotoxin, 4'-demethyl-, 4,6-O-ethylidene-β-D-glucopyranoside	9/15/72		
404,241	Adenine, 9-β-D-arabinofuranosyl-	1/27/72		
	Total IND's Filed (Non-endocrine)		5	8

Compiled by Eleanor M. Sloane
Comparison provided by Ruth Park

Table II

Distribution of PRA Monitored Contract Projects by DCT-Array[1]

Array	Linear L	Supplementary S	Exploratory E	Total
IXX	207,100	542,400	0	749,500
2AX	353,100	0	0	353,100
2B2	198,200	0	0	198,200
8XX	38,000	113,000	0	151,000

		Project	(Amount, $)
L-1XX	DR&D Chemical Information System	-- operation, registration of new agents	129,400
L-1XX	DR&D Biological Data Processing System	-- operations, acquisition data	62,100
L-1XX	DR&D Biological Data Processing System	-- improvement, acquisition data processing	15,600
S-1XX	DR&D Chemical Information System	-- development	542,400
L-2AX	DR&D Biological Data Processing System	-- operations, antitumor activity data	272,700
L-2AX	DR&D Biological Data Processing System	-- improvement, antitumor activity data processing	80,400
L-2B2	DR&D Biological Data Processing System	-- operation, detailed evaluation data	115,300
L-2B2	DR&D Biological Data Processing System	-- improvement, detailed evaluation data processing	82,900
L-8XX	Drug Distribution & Inventory Data System		38,000
S-8XX	Cancer Chemotherapy Abstracts preparation		113,000
	TOTAL PRA MONITORED PROJECTS		1,451,800

[1] Funding based on amount negotiated for FY 73, if any, or on amount obligated in FY 72 as of January 31, 1973. DCT Monthly Report of Contract Commitments.

Table III

PRA Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Program Analysis Branch	Funding of Tasks Monitored by Program Analysis Branch Distribution by Project (Code)	(%)	($)
CHEMICAL ABSTRACTS SERVICE (65-1021)		100	129,400
DR&D Chemical Information System -- operation	L-1XX	100	129,400
HERNER INFORMATION SERVICES (72-3244)		100	113,000
Cancer Chemotherapy Abstracts -- preparation	S-8XX	100	113,000
LITTLE, ARTHUR D. (72-3257)		100	120,000
DR&D Biological Data Processing System -- improvement in processing acquisition data	L-1XX	13.0	15,600
DR&D Biological Data Processing System -- improvement in processing antitumor activity data	L-2AX	67.0	80,400
DR&D Biological Data Processing System -- improvement in processing detailed evaluation data	L-2B2	20.0	24,000
PENNSYLVANIA UNIVERSITY (71-2187)		100	50,000
DR&D Chemical Information System -- design	S-1XX	100	50,000
PENNSYLVANIA UNIVERSITY (73-3719)		100	492,400
DR&D Chemical Information System -- implementation	S-1XX	100	492,400
SOUTHERN RESEARCH INSTITUTE (71-2098)		2.8	58,900 2,131,400
DR&D Biological Data Processing System -- improvement in processing detailed evaluation data	L-2B2	2.8	58,900

Table III (Continued)

PRA Contractors. Distribution of Effort Among Projects

CONTRACTOR (Contract Number) Projects Monitored by Program Analysis Branch	Funding of Tasks Monitored by Program Analysis Branch Distribution by Project (Code)	(%)	($)
THOMPSON, JOHN I (69-58)		100	38,000
Drug Distribution & Inventory Data System	L-8XX	100	38,000
VALUE ENGINEERING (73-3706)		100	443,600
DR&D Biological Data Processing System -- operation processing acquisition data	L-1XX	14.0	62,100
DR&D Biological Data Processing System -- operation, processing antitumor activity data	L-2AX	60.0	266,200
DR&D Biological Data Processing System -- operation, processing detailed evaluation data	L-2B2	26.0	115,300
VITRO LABORATORIES (69-58)		100	6,500
DR&D Biological Data Processing System -- operation, processing antitumor data (terminal)	L-2AX	100	6,500

Table IV

PRA Projects, Distribution of Effort Among Contractors

PROJECT DESCRIPTION	CONTRACT (Contract No.)	FUNDING ($) (Negotiated FY 73 or obligated FY 72)	
DR&D CHEMICAL INFORMATION SYSTEM--OPERATION		L-1XX	129,400
Registering the structure and nomenclature of new agents and searching for analogs	Chemical Abstracts Service (65-1021)	129,400	
DR&D CHEMICAL INFORMATION SYSTEM--DESIGN		S-1XX	50,000
Designing an improved system for the registration, control, shipment and inventory of agents	U. of Pennsylvania (71-2187)	50,000	
DR&D CHEMICAL INFORMATION SYSTEM--IMPLEMENTATION		S-1XX	492,400
Implementing the design for the improved system	U. of Pennsylvania (73-3719)	492,400	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--OPERATION, ACQUISITION DATA		L-1XX	62,100
	Value Engineering (73-3706)	62,100	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--IMPROVEMENT, ACQUISITION DATA		L-1XX	15,600
Development improved techniques for processing acquisition data for new agents	Little, Arthur D. (72-3257)	15,600	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--OPERATION, ANTITUMOR ACTIVITY DATA (TERMINAL)		L-2AX	6,500
	Vitro Laboratories (69-58)	6,500	

Table IV (Continued)

PRA Projects, Distribution of Effort Among Contractors

PROJECT DESCRIPTION	CONTRACT (Contract No.)	FUNDING ($) (Negotiated FY 73 or obligated FY 72)	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--OPERATION, ANTITUMOR ACTIVITY DATA		L-2AX	266,200
	Value Engineering (73-3706)	266,200	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--IMPROVEMENT, ANTITUMOR ACTIVITY DATA		L-2AX	80,400
Developing improved techniques for processing antitumor activity data	Little, Arthur D. (72-3256)	80,400	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--OPERATION, DETAILED EVALUATION DATA		L-2B2	115,300
	Value Engineering (73-3706)	115,300	
DR&D BIOLOGICAL DATA PROCESSING SYSTEM--IMPROVEMENT, DETAILED EVALUATION DATA		L-2B2	82,900
Developing improved techniques for processing detailed evaluation data	Southern Research (71-2098)	58,900	
	Little, Arthur D. (72-3256)	24,000	
DRUG DISTRIBUTION AND INVENTORY DATA SYSTEM		L-8XX	38,000
	Thompson, John I. (69-2158)	38,000	
CANCER CHEMOTHERAPY ABSTRACTS--PREPARATION		L-8XX	113,000
	Herner Information Services (72-3244)	113,000	

Drug Shipments and Inventory

The Drug Cost Reporting System was expanded this fiscal year to function as a full drug inventory as well as a cost report of drugs shipped. In the past, this computer system recorded the cost of commercial and investigational drugs shipped in total, and by such variables as drug name, drug lot number, investigator, protocol, study group, NIH staff, clinical and non-clinical use of drugs, and other stratification. In addition, the expanded system keeps track of the quantity of each lot in inventory, the expiration date and re-order level of each lot, the bulk drug costs, and the formulation costs. These reports flag the need for re-order, and incidentally emphasize the heavy use of some drugs and monitor expiration dates (shelf-life) of all drugs in stock. Considered in the need for re-ordering is the time necessary to manufacture and formulate the drug, because the lag-time between re-ordering and receipt of drugs varies.

New report formats were added and some of the older, more detailed reports were summarized. The most important additions were the actual inventory reports which are produced daily and the summarization of detailed reports.

The standard drug cost reports include:

(a) A monthly report of drugs shipped to cooperative groups broken down by NSC number and a similar report broken down by investigator within the cooperative group.

(b) A monthly report of all drugs shipped from DR&D broken down by NSC number, lot number, and dosage form.

(c) Quarterly reports which include the two reports mentioned above as well as breakdowns of drugs supplied to independent, intramural, and selected investigators. (A one-page summary of this report is also available.)

(d) A semi-annual report which consists of a one- or two-page report for each investigator having received drugs in the past six months. It includes the quantity and cost of every individual NSC number and lot number shipped to a particular investigator. Accompanying this report is a slightly less detailed breakdown for the use of the group chairman.

Important additions have been monthly reports detailing the distribution of adriamycin and bleomycin to cooperative groups, independent investigators, and intramural investigators. A one-page summary of these reports is avail-

able as well. The bleomycin report also includes a monthly projection of expected costs for the ensuing 12 months.

As a part of the US-USSR Agreement, a number of drugs are now being sent to the Soviet Union by the Division of Cancer Treatment. These shipments are being routinely identified with a group code of "RUS," and appear separately in the monthly and quarterly reports.

The standard inventory reports are:

(a) A daily report flagging all drugs needing to be re-ordered.

(b) A daily report flagging all lots nearing expiration.

(c) Monthly inventory listings.

The investigator address file was re-implemented this fiscal year after two years of inactivity. Mailing labels are produced selectively from this file, for example, to notify all users of a given drug of an adverse reaction; to notify group chairmen of any action; or to notify users that a drug is now to be obtained commercially. The complete address listing is also used as a reference by the Cancer Therapy Evaluation Branch. Re-implementing this file entailed making 14,000 updates to the old file and writing new programs to maintain the file and produce address listings and mailing labels.

There was an increased demand for special queries of the drug shipment files. A typical request is to report on all nucleosides shipped during the past three fiscal years. These included listings of all shipments of specified compounds; reports of shipments to selected investigators; month by month drug cost analyses to predict future demands; investigator address listings; and investigator mailing labels. For the most part, response time for these queries stayed well under 48 hours and high-priority requests were filled in 2-5 hours.

The cost of designing a new report averaged about $35.00. The cost of re-running a previously written query generally stayed between $6.00 and $15.00, depending on the complexity of the selection and the number of files accessed. Producing mailing labels was more expensive. The cost varied from $6.00 to $60.00, depending once again on the complexity of the selection and the number of labels printed. The cost per label decreased significantly as the number of labels produced increased.

A small on-line computer system was developed to replace the Kardex system in the Drug Liaison and Distribution Section of Cancer Therapy Evaluation Branch. This system will provide instantaneous information on NSC number, lot number, dosage form, quantity on hand, manufacturer, and expiration date, which will greatly facilitate their daily operations. The Kardex system required hand filing, clerical search, and manual posting in the daily operations of the Drug Liaison and Distribution Section. The on-line system eliminates this and is referenced simply by NSC number and drug formulation. This new system will obviate the need for an additional clerk typist in CTEB at approximately the same annual operating cost.

OFFICE OF THE ASSISTANT CHIEF

Program Analysis Branch

DR&D Biological Data Processing System:

Major responsibility for the DR&D biological data processing system remained in the Office of the Assistant Chief during calendar year 1972. The integrity of the file continued to be upgraded, and the types of data processed were expanded. Modifications were made in the processing of the file which enhanced its efficiency and usefulness. In July 1972, the Data Processing contractor changed from Vitro Laboratories to Value Engineering Company. This caused a slow-down in the processing of data due to the transfer of all materials, data sets, programs, procedures, and documentation, and to the necessary retraining of contract staff. By the end of calendar year 1972, all of the functions of the data processing system were in a "go" situation with some improvements made in both programs and flow as the work was taken over by the new contractor. As a part of this transfer all of our programs were modified to run at DCRT. In some cases this required re-writing portions of our entire programs.

The following reports were produced and distributed routinely, schedules being interrupted only during the transition period when the data processing contractor was being changed:

Routine System Output:

- Screening Data Summaries - this schedule was never interrupted; about 3,000 per week were mailed to suppliers and screeners;
- Screening Experimental Analysis Reports
- Interim Screening Transaction Reports
- Address Labels for mailing
- Combination Chemotherapy Reports

DR&D Program Management Aids:

- Control Pack Index - index to microfilm of raw test data
- Material Shipping List Index - index to microfilm of raw shipping data
- Quarterly Statistical Report
- Forms Control Report
- Combination Chemotherapy Cross Reference Report
- Selected Agents Compounds (SAC) Report

DEB, Screening Program Management Aids:

- Control Performance Report
- Control Line Listing
- Weekly Statistical Report by Special Study Code
- Missing Control Pack Report
- Schedule Dependency Report
- Status Code 15, 20C, 26P, 27P, 28P Reports (Confirmed Active Testing)

DDB, Natural Products Management Aids:

- Materials of Interest Report (Natural Products)
- Confirmed Plant and Animal Report
- Plant Header Reports (Botanical Names)
- New Plant Extracts (Fractionator)
- Quarterly Fractionation Report
- Cross Index to Plant Header File

In addition to routine reports, 57 special queries were programmed during calendar year 1972 including:

- Plant and Animal Names by geographic location for collection teams
- Materials supplied by grantees
- Materials not tested in specific systems (for use by Screening Section in scheduling materials for new protocols)
- Comparisons of tests versus materials confirmed for planning screens
- Number of BDF_1 and CDF_1 mice used in a particular period
- Active Baker compounds
- Biological data for antifols

The Plant Header File was microfilmed for the first time in February 1972. The file had grown in size to a point where it was impossible to use the printed reports any longer. In October 1972, it was microfilmed at Division

of Computer Research and Technology (DCRT) by Computer Output microfilm (COM) for the first time. This obviated the need for printing the file prior to microfilming (a process that consumed 10 boxes of computer paper), reduced the cost of the procedure, and reduced the time for preparing the microfilm. The Plant Header file has now grown to 79,680 entries: 72,850 plant materials and 6,830 animal materials. Cumulative microfilm is prepared quarterly; additions and modifications are printed monthly. It is prepared in three different sort orders: by family, by genus and species, and by NSC number.

A file containing botanical names of plants which have been assigned a Material Classification (MC) code of C, D, U or V or have been collected more than 9 times has been created and is updated regularly for the Natural Products Section, DDB. A program to access this file from a remote station was written by Michael Shamos during his years with PRA.

The DDB uses this file and program to determine which plants should be sent to a contractor for extracting and which can be excluded.

The program and file are presently maintained by PRA staff who are in the process of updating this file from the Materials of Interest (MOI) report. As a by-product of this program a list of plants and the number of times they have been collected is sent to the Natural Products Section regularly. Dr. Perdue, of the U.S.D.A., is ordering a remote terminal so that his staff may carry out this function and avoid sending unwanted plants to Wisconsin and the handling involved at both ends.

The Quarterly Fractionation Report, prepared for the Natural Products Section, was expanded to include a Summary Fractionation Report. This report had previously been produced by hand from the longer report. This has saved statistical clerk and secretarial time (in short supply) and has made the report available on a more timely basis for management control.

The backlog of and current biochemical data (Dihydrofolate Reductase Inhibition and Millimolar Concentration which achieves 50% Inhibition in Selected Test Systems and Selected Tumors) was entered into the system during calendar year 1972. It has been available for queries and has been a part of the normal Screening Data Summary output since June 1972.

Programming changes were incorporated into the system to allow solid tumor studies for tumors evaluated by mean and median tumor weight estimated from diameters. This data is now being entered into the system routinely. Along with the present data, backlogs of solid tumor data are also being entered for future retrieval and comparison.

Last year's annual report noted the creation of a schedule-dependency summary file and an on-line computer system which allowed retrieval of the summaries by NSC number. This system was expanded to allow retrieval by screener/control number as well. Additional programs were written to add data directly to the on-line summary file from the cards produced by the Biological Data Processing system. Originally, these cards were used only as input to the schedule-dependency plotting program. This plotting program was also modified to accept all test systems. The old version of the program only plotted L1210 data.

Combination chemotherapy testing received for data processing has risen dramatically during 1972. In total, over 5,000 tests were encoded and are in the process of being added to a separate file maintained for this data. Some of this testing represents backlogs from Microbiological Associates, Inc., and Southern Research Institute which are being added to the file for future storage and retrieval.

In conjunction with the reports describing individual combination experiments, a cross reference report of compounds tested together and the test system used is produced as routine output of this system. This report has recently been modified to include the chemical names of the compounds in the system. In addition, the AKR spontaneous tumor combination testing performed at Southern Research Institute has been added to this report for ready reference.

During 1972 the Office of the Assistant Chief monitored and helped in the development of work performed under contract at Southern Research Institute.

Two programs comprised the major part of this work. The system that stores and reports on AKR data was developed further to include statistical and graphical data summaries. The toxicity system was enlarged to include new report programs and a technique was developed for analyzing and reporting on the combination chemotherapy data.

DATA PROCESSING AND COMPUTER SYSTEMS DEVELOPMENT SECTION

Program Analysis Branch

During FY 1973 the Data Processing and Computer Systems Development Section concentrated on completing the design and starting the implementation of the DR&D extended chemical information system which will replace the current semiautomated system.

Efforts were made to maintain compatibility with the Chemical Abstracts Service (CAS) system to benefit from improvements arising out of CAS developments, including such capabilities as the mechanized identification of new accessions in the DR&D system with unique ring systems or compounds in the literature with antitumor activity or significant activity in other biological systems. Abstracts of Cancer Chemotherapy Abstracts were captured on computer tape in a format suitable for possible inclusion in the International Cancer Research Data Base. In support of the compound acquisition effort, the Section continued to experiment with a variety of mechanized methods to identify analogs of compounds of interest to the Program reported in the literature or available in industrial collections.

A. Support of the Linear Array

This Section supports the DCT Decision Network by monitoring the development of the DR&D extended chemical information system to assist in the acquisition of new agents (L-1XX). In addition, the Section demonstrated the feasibility of using computer tape services and data bases of commercial organizations to identify for acquisition analogs of compounds of interest which were reported in the literature or newly synthesized or in industrial collections.

B. Design of the DR&D Chemical Information System

Under contract, the University of Pennsylvania completed the detailed design of the DR&D extended chemical information system in November 1972. The decisions made on a variety of technical problems and the complex chemical or computational elements of the design are fully documented.

Extensive consultation was provided during the design phase by designers and operators of large chemical information systems in government, industry, and non-profit institutions so that the Section could benefit from their experience and could incorporate into the design proven developments in the state-of-the-art. Organizations which furnished particularly valuable assistance included CAS, Walter Reed Army Institute of Research (WRAIR), Edgewood Arsenal, Eastman Kodak, and the Division of Computer Research and Technology (DCRT), NIH.

To assure that the design met user requirements, presentations were made to DR&D as critical milestones were reached or products delivered. For example, presentations were made on the overall functional design, the detailed functional design, the overall work flow plan, and the design specifications. Computational and chemical information specialists exchanged information pertinent to the design specifications. Comments which improved the system were incorporated into the design.

In designing the system, user needs were carefully reviewed against cost considerations and decisions made to defer implementing specific system features requested by users until technological advances would permit incorporating these features into the system with acceptable costs.

C. Implementation of the System

In November 1972, the University of Pennsylvania, under contract, commenced an incrementally funded, phased effort to implement the design of the chemical information system. The system will control the flow of synthetic compound samples in the acquistion process through 23 possible work stations and eight distinct journeys or routes through various combinations of these stations. When the system is fully operative, samples will be ready for shipment to screeners within one week from receipt by DR&D.

The system will relieve the staff of many tasks now done manually and will provide mechanisms for improved management control of the Program and more fruitful use of materials and data. Specifically, the system will record structural information for new submissions to the Program, along with their sources and other data; determine whether the submissions duplicate previously tested compounds; and accordingly schedule testing and initiate shipment to screeners. Submissions that are duplicates or are closely related to known active compounds will be automatically brought to the attention of management or automatically assigned to specialized testing procedures by programmed decisions. The system will maintain an inventory of materials on hand, permit on-line inquiry for quantities available for shipment, support replenishment procedures, and control the distribution of rare or expensive compounds. Routine automated outputs will include acknowledgment to suppliers of the receipt of submissions, chemistry cards (including structures and names), Material Shipping Lists, bottle labels, requests for declassification or replenishment of supply, etc. Operational control reports will alert a System Manager of the need for remedial action to accelerate the flow of materials to the screeners.

Management reports will include the following: (1) sample submissions by supplier over time and comparisons with submission rates of previous periods; (2) acquisitions by type of supplier or acquisition method; (3) sample size statistics; (4) summaries of requests by investigators outside the Program; (5) compounds having both open and discreet samples; and (6) compounds submitted under grants or contracts.

An inquiry part of the system will permit a biologist or chemist, for example, to access the files using simplified inquiry procedures for full structure search (to secure an immediate answer for a possible duplicate sample) and substructure search (for analogs). Retrieval of non-structural data (suppliers, NSC numbers, inventory, status in the Linear Array, shipments, etc.) will provide rapid response to management information needs.

The implementation and installation of the new system is a complex effort requiring the timely integration of many developmental tasks and coordinating the support of independent organizations such as CAS and DCRT. To assure the delivery of system components, Program Evaluation and Review Techniques (PERT) are employed to monitor the performance of the contractor and to identify quickly and resolve problems which potentially could delay the installation of the system. Regularly scheduled Project Officers' meetings attended by key DR&D staff members and NIH consultants are used to resolve technical issues which affect the users of the system.

D. Operation of the System

DR&D's new chemical information system will be operated by a support contract in FY 1974. The contractor must possess a combination of technical and managerial skills to operate this computer-based, chemical structure and nomenclature system.

E. Mechanized Methods for Compound Acquisition

Experiments to identify compounds reported in the literature or in private collections of interest to the Program were continued during FY 1973 with the cooperation of the DDB and the use of more rigorous criteria of interest.

1. Institute of Scientific Information (ISI)

Highly specific moieties of interest, selected from drugs in general practice, undergoing clinical trials, or in preclinical toxicology, were used for analog searches of the ISI computerized files. These files report newly synthesized materials in the fields of organic, biochemical, bioorganic, pharmaceutical, and agricultural chemistry. Twenty-one queries searched against three monthly tapes yielded 256 valid analogs in such classes as anthroquinones, macrolide antibiotics, harringtonine

alkaloids, vincristine analogs, and rifamycin analogs. These analogs are being automatically duplicate-searched against DCT's Registry File to determine whether the 5% duplication rate experienced in an early study will be repeated. Information for 219 analogs (85% of the total) were reported in articles by investigators in 29 foreign organizations, only one of which is a supplier to the Program. Articles by investigators in Europe were made available to the Brussels Liaison Officer for acquisition purposes. Articles by investigators in the eight domestic organizations (including one which is not a supplier) will be turned over to the acquisition contractor after duplicate checking. Aside from the potential of acquiring samples of analogs of interest, this selective acquisition approach may develop new suppliers, including investigators from foreign countries, and provide means of maintaining a dialogue with existing suppliers. If these acquisition efforts are successful, a retrospective search could be made of the ISI files (begun in 1967) which include 900,000 compounds reported as newly synthesized. This search could be broadened to identify all compounds with reported biological activity.

2. Shell Development Company

With the cooperation of information specialists in the Shell Development Co., 16 of the above 21 searches made against the ISI files were also automatically searched against Shell's file of 30,000 compounds. A total of 269 analogs were reported for five classes, including quinones, phenothiazines, and triazoles. The structural diagrams that were output by the Shell automated system are being duplicate-checked against our Registry File. DDB will request samples for compounds either not in our collection or for which an additional supply is needed.

3. Eastman Kodak

With the cooperation of information specialists at Eastman Kodak and CAS, and at a cost of less than a penny per compound, the structures of 5,800 open Kodak compounds on a computer tape were automatically searched for duplicates in our collection. Of the 42% which were not duplicates, 645 were identified in the Kodak catalog. These were reviewed for Program interest and acquisition.

4. Other Acquisition Support

Relationships with information specialists in government and industry were used to initiate and increase the contribution of samples to the Program. A dialogue covering a span of several ACS national meetings contributed to the

significant submission of samples by Rohm and Haas during FY 1973.

F. Experimental Machine-Searching of Cancer Chemotherapy Abstracts

In cooperation with the Literature Research Section, experimental comparison of automated text searching with the manual method was made using one year of Cancer Chemotherapy Abstracts (CCA) as a data base. Neither method demonstrated significant superiority in this experiment. However, with the addition of index terms on the file and positional search capability, the mechanized system should prove more effective.

As a byproduct of publishing CCA, the abstracts and CCA index terms are being captured on computer tapes. These tapes for 1971 and 1972 together with abstracts in a machine-processable form from 1967 through 1970 are being retained for possible inclusion in the International Cancer Research Data Base.

G. Miscellaneous

The Section participated in a feasibility study by J. I. Thompson and Co. to analyze the need for an information system to handle the management and scientific information available in contract reports. A report outlining the recommended system to meet the information need was submitted.

The Section also coordinated the collection of information required by NIH, DHEW, and GSA for the Automated Data Processing and Management Information Reporting System.

During FY 1974, the Section will concentrate on: (1) completing the implementation of the Phase I chemical information system; (2) developing a contractor to operate the new system; (3) installing the new Phase I system; (4) and beginning the implementation of the Phase II system. Increased efforts will be made to exploit literature data bases and automated files of compound collections to acquire samples of special interest to the Program.

LITERATURE RESEARCH SECTION

Program Analysis Branch

The Literature Research Section serves the Cancer Treatment Program by providing information on all forms of cancer treatment. References and materials are obtained from the fields of surgery, radiotherapy, immunotherapy, and chemotherapy and the related chemical and biochemical disciplines. Computer services provided by the National Library of Medicine and the Division of Computer Research and Technology aid in the literature searching. Primary responsibilities include the gathering of information for use in Decision Network Review, for meeting FDA requirements for IND filing, and for evaluation of clinical studies.

Literature Services. Information obtained from the world's published literature and from NCI Contract Progress Reports is provided in the form of abstracts, bibliographies, computer print-outs, and copies of articles. More than 200 requests were filled during the year. Bibliographies were prepared, or updated, for such agents as streptozotocin, neocarzinostatin, ellipticine, CCNU, methyl-CCNU, arabinosyl adenine, cyclocytidine, cytembena, hycanthone, thalicarpine, daunomycin, and adriamycin. Extensive clinical searches were performed for the chemotherapy of thyroid and endometrial cancer; the chemotherapy of chordomas and sarcomas; the surgery, radiotherapy, and surgical adjuvant treatment of pancreatic carcinoma; the natural history, etiology, and epidemiology of colon and stomach cancer; and the radiotherapy and chemotherapy of colon cancer.

Through an agreement with the National Library of Medicine, the Section is coordinator of the National Cancer Institute's access to the Library's on-line automated bibliographic search system, MEDLINE. In this role the Section serves all areas of the Institute, providing assistance in the processing of search requests and instruction in the use of the terminal. During the first nine months of operation, the staff assisted over 35 investigators, many of whom are outside the Division of Cancer Treatment. Ready access to citations in the MEDLARS data base greatly facilitates the answering of requests and the maintenance of the Section's files on agents and human cancers. Searches are performed monthly to update the clinical files and to aid the continuing search for new and potential antineoplastic agents. Evaluation of other available automated selective dissemination of information services continues, using CBAC tapes at DCRT and CA Condensates at the IIT Research Institute. A current awareness service utilizing title search only was dropped when it proved inadequate for the needs of the program.

The preparation and distribution of the agent folders for the Decision Network Meetings is a continuing responsibility. Each folder contains the compound's structure and systematic name, an analog search, a literature search, summaries of clinical or preclinical data, and questionnaires. During the year, materials were assembled for a total of 44 drugs.

The Section is also responsible for compiling and distributing the Division of Cancer Treatment Program Statistics. Data were assembled and cumulated quarterly for the number and types of materials entering the Drug Research and Development Biological Data Processing System, the tests performed, the number and types of confirmed actives in the primary screen, the compounds passing each Decision Network step, including criteria for 2A passage, and the drugs for which INDs were filed.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library. Copies of over 100 biomedical journals are regularly received in the library in addition to the abstracting and indexing services of Chemical Abstracts Service, the National Library of Medicine, the Excerpta Medica Foundation, and the Biosciences Information Service of Biological Abstracts. Selected monographs and a file of current NCI Contract Progress Reports are also available for the use of the Program staff. Over 1700 journals were circulated during the year and approximately $12,000 was spent on books and journal subscriptions.

Contract Operations. To provide the cancer investigator with abstracts from the world's literature on cancer chemotherapy, members of the staff monitor the contract for the preparation of Cancer Chemotherapy Abstracts. Every effort has been made this year to bring this journal up-to-date and to improve its appearance and organization. The subject sections, particularly those dealing with clinical studies, have been refined and broken down to facilitate scanning and searching. Consideration is being given to expanding the coverage to include surgery, radiotherapy, and immunotherapy of cancer. As by-products of the contract, the Section receives computer tapes of the abstracts and indexes and hard copy of all articles cited. The feasibility of utilizing the tapes for automated text searching is being explored.

PUBLICATIONS SECTION

Program Analysis Branch

The Publications Section directs the preparation of Cancer Chemotherapy Reports (CCR), the principal scientific journal developed under the DCT Program, officially clears all DCT contract manuscripts, and provides editorial assistance to the DCT staff on a time-available basis. Until 1968, CCR was a single journal but in that year it was expanded to 3 parts. From its inception, CCR (and CCR Part 1) has published original contributions in clinical and experimental studies of cancer chemotherapy, including complete works, preliminary investigations, commentaries and reviews, symposia, and letters pertinent to published articles. All candidate manuscripts are reviewed by 2 or 3 investigators familiar with the subject matter and the final determination of acceptance or rejection is made by the Scientific Editors. CCR is now in its 15th year of continuous publication with a total to date of 96 issues. In 1972, there were 6 issues of CCR Part 1 (Vol. 56, 814 pages). Six issues of Part 1 are planned for 1973.

CCR Part 2, which contains chemotherapy screening data, structure-activity relationships, and other related material, has been published since 1968, as manuscripts and personnel have been available. The fourth and most recent issue, Vol. 3, No. 1, was published in November 1972 (340 pp). Two issues of Part 2 are planned for 1973.

There have been 5 issues to date of CCR Part 3, which contains non-reviewable program information. The second and third of these issues, the Proceedings of the First and Second Joint Working Conferences, NCI Chemotherapy Program, were published in October 1971 and May 1972. It is anticipated that the Proceedings of this conference will be published annually in CCR Part 3. The Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems (Third Edition) were published in September 1972 and an issue containing clinical brochures, toxicology reports, and the Protocols of the Laboratory of Toxicology was published in January 1973. Four issues of Part 3 are planned for 1973.

In 1973 CCR will be published monthly for the first time since its inception. Part 1 will appear bi-monthly starting with the February issue, and an issue of either Part 2 or 3 will be published during the remaining 6 months. Assuming the current trend of manuscripts received continues, monthly publication will be a permanent feature of CCR.

In FY 72, several new features were added to CCR, such as the inclusion of authors' addresses in the Contents, the use of CAS registry numbers to identify all compounds and agents, and a new section entitled Brief Reports and Preliminary Communications. Authors were invited to submit short clinical notes or the abbreviated results of preliminary work to this section. It is anticipated that these reports will be processed through the editorial channels more quickly than a full manuscript requires, thus allowing an early publication date. The response to this new section has been excellent during the first 2 months of 1973.

CCR has continued to expand its scope of scientific coverage during the past year. Parts 2 and 3 will be increasingly used to disseminate such information as the Proceedings of meetings (eg, the Workshop for Therapy of Lung Cancer, held in October 1972, and the Screening Contractors Conference to be held in March 1973) and study protocols which are used widely in this country and many foreign countries. During FY 73 CCR was asked by the American Society of Clinical Oncology to publish the Abstracts of Reports presented at their Annual Meeting. These abstracts will be published each year in the February issue of Part 1.

Comparative data on the CCR series of publications are shown in Table 1. The manuscripts that are accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports.

The Publications Section is exploring ways in which to speed up the publication time of CCR Part 1. Although certain absolutes exist in the printing process for all journals (which must be handled through the GPO), the streamlining of existing editorial procedures could effect noticeable changes in the publication time for this journal. During FY 73 many duties previously assigned to editors have been delegated to editorial assistants. This experiment has proved valuable, allowing editors to concentrate entirely on editing functions and affording editorial assistants the chance to handle more challenging assignments. With the proper controls and review processes, this shifting of duties may expedite more rapid publication time.

The Publications Section currently has 2 full-time editors. During FY 73 the Section has been working with a Project Stride trainee. The trainee works 20 hours a week, learning the techniques and skills of a technical publications editor, and attends American University the other 20 hours, working toward a BA degree. Her job experience is intended to supplement her studies at the University. Upon graduation (in 1975) the trainee will not only have a degree but will have had a great deal of job experience, and hopefully she will remain in the Publications Section as a full-time editor. Her assistance to the production of the Section has been valuable; the program appears to be an excellent one, benefiting both the trainee and the supervisor.

Since 1967 CCR has been listed in Current Contents, Life Sciences. In February 1973, CCR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in Europe.

In 1964, CCR began sending corrected page proof to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During FY 73 CCR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia. This trend toward more extensive coverage of CCR indicates a growing interest in the field and in CCR in particular.

The Publications Section is also responsible for maintaining the free mailing list for CCR. CCR is available without cost to qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3710	Foreign and domestic free mailing (includes ca. 175 at NIH plus storage copies for Section)
500	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depository Libraries)
Total 4577	

Problems with the service rendered by the GPO in maintaining the mailing list have brought about a change in the distribution procedures. Beginning in February 1973 the mailing list will be transferred to the Distribution Section, Printing and Reproduction Branch, ODA, NIH. This conversion will allow more rapid service in 2 areas: (a) adding, deleting, and changing names on the list, and (b) obtaining mailing tapes to be used by the contract printer in mailing the issues to the subscribers. It is estimated that NIH will be able to perform these functions in one fourth of the time GPO requires.

Another function of the Section is the coordination of clearance of DCT contract manuscripts for publication. In CY 72, 166 of these manuscripts were cleared, and during the first month of CY 73, 31 manuscripts were cleared. In assuming the responsibility for clearance of these manuscripts, the Publications Section checks all NSC numbers mentioned in the papers, and notifies the DDB when compounds must have publication clearance from suppliers. The authors are instructed to reword footnotes as necessary to give proper credit to the DCT. The Section ensures that the Project Officer sees all papers and signs the correct forms. Records and reprints are kept for all manuscripts cleared, and quarterly reports are prepared for distribution to staff personnel.

Table 1.--Summary data on the CCK Publications Series

	CY 69	CY 70	CY 71	CY 72	CY 73 (Jan 1-Feb 9)
Mss. received	78 (pt 1) 4 (pt 2) 6 (pt 3)	122 (pt 1) 5 (pt 2) 1 (pt 3)	107 (pt 1) 6 (pt 2) 11 (pt 3)	122 (pt 1) 4 (pt 2) 47 (pt 3)	11 (pt 1) 0 (pt 2) 17 (pt 3)
Mss. accepted	63 (pt 1) 5 (pt 2) 6 (pt 3)	68 (pt 1) 4 (pt 2)	73 (pt 1) 5 (pt 2) 11 (pt 3)	76 (pt 1) 5 (pt 2) 47 (pt 3)	8 (pt 1) 0 (pt 2) 17 (pt 3)
Issues published	6 (pt 1)	6 (pt 1)	5 (pt 1) 1 (pt 2) 1 (pt 3) 7	6 (pt 1) 1 (pt 2) 2 (pt 3) 9	1 in press (pt 1) 1 in press (pt 3) (12 issues planned)
Pages published	408 (pt 1)	492 (pt 1)	621 (pt 1) 246 (pt 2) 104 (pt 3) 971	814 (pt 1) 353 (pt 2) 181 (pt 3) 1348	150 in press (pt 1) 150 in press (pt 3) (1800 pp planned)
Reviewers	132	140	164	197	55
Printing costs*	$11,813 (28.95/pg)	$15,334 (31.16/pg)	$33,284 (pt 1) (53.59/pg) $14,828 (pt 2) (60.27/pg) $4,564 (pt 3) (43.88/pg)	$30,000† (pt 1) $10,000† (pt 2) $ 8,000† (pt 3)	Not available

* Costs of GPO contract with Publications Press, Inc., Baltimore, Md.

† Estimated costs, actual figures not available March 1, 1973.

SCIENTIFIC RECORDS SECTION

Program Analysis Branch

The Scientific Records Section (SRS) stores, analyzes, and dispenses the majority of the chemical information used in the development of the Division of Cancer Treatment's drug research program. The Section provides supporting services to all major areas of the Program and during the past year has been modifying its functions to supply more analytic data. In order to fully utilize the files of chemical and biological data that have been built in the past 18 years, the Section has the ability to search the chemical file by structure or substructure and to provide data such as structural formula, systematic name, and biological data in various formats to requesters. All data for structure-activity studies are gathered in this Section with the cooperation of other sections in PRA, and new methods are continually investigated to facilitate such correlation.

The Section prepares and maintains the master structure file and several cross-reference files on each drug. It indexes and organizes new compounds into files suitable for retrieval by name, NSC number, molecular formula, and chemical substructure. Section personnel also prepare shipping lists for the transmittal of compounds to screeners and coordinate the various special shipping instructions from DR&D for certain categories of agents. During the past year the Section has taken over full responsibility for the structuring and naming of new acquisitions.

As biological data are returned by screeners, Section personnel monitor some aspects of its quality and verify its entry into computer files. Screening data for both natural products and synthetic compounds are sorted, checked, and filed, and requests for copies of specific data are filled from film or paper, or by computer inquiries. The production of several monthly status reports on active synthetic and natural products is coordinated by Section personnel.

Section personnel advise on problems in the biological data processing system and aid in the implementation of solutions.

Chemical File

A. Registry of New Compounds

The structures of all new compounds received in the Drug Development Branch (DDB) are drawn by the SRS according to fixed conventions on a 10 x 8 card. These cards are sent weekly to the Chemical Abstracts Service (CAS) for determination of duplicates and updating of the master computer chemical file. When the cards are returned to SRS, new compounds are assigned systematic nomenclature and all compounds are given to DDB for assignment of NSC number and notification of the supplier. SRS then prepares shipping lists for use as transmittal documents to accompany compounds to the screener and sends NSC and

nomenclature information to CAS for input to the name file.

The volume of compounds increased significantly in calendar year 1972 with the advent of a collection contract and several large purchases. Of the 20,308 compounds acquired in 1972, 16,480 were structured in SRS and the new compounds named.

In a cooperative effort with Drug Evaluation Branch (DEB) to retest all duplicate compounds in present testing protocols, screening data for all duplicate compounds were obtained by computer inquiry by SRS, and structures, amount received, and biological data were sent to DEB for determination of screening adequacy and shipment for retesting.

B. Shipping

The Section prepared shipping lists for 13,201 compounds in calendar year 1972 and maintained and updated the master screener and supplier address files using a card system and an interactive computer system. In October 1972, Section personnel cooperated in the development and implementation of a priority assignment of NSC numbers and shipping procedure for all contract compounds. It was hoped that bypassing a good deal of the initial cataloging process would allow quicker return of data to contractors of DR&D and thus facilitate targeted synthesis programs. Compounds from synthesis contractors were assigned NSC numbers without going through the normal route of machine duplicate check and naming, thus saving the time spent in this process. These compounds were shipped to a single screener assigned to give them priority testing, and the raw data was sent to the supplier for initial notification of results prior to the final computer-produced report. The system did result in faster return of data to synthesis contractors although there were some disruptions in the flow of chemistry cards between the manual and computer files.

C. Name and Formula Indexes

Systematic names and synonyms, plus supplier name and discreet and publication codes, are stored in a text file at CAS. Quarterly indexes sorted by molecular formula and alphabetically by name are computer-produced.

During the year an effort was completed to update and correct the information on this file for discreet, publication, and supplier code status for each compound. A program was written to accept the "1D" correction cards used in the biology files and convert them into a format suitable for the name file. This correction run used input from a past cross-checking of the biology and name files as well as recent declassification of compounds. The run successfully corrected these data for over 6,600 compounds.

In addition to corrections, additional CAS registry numbers were added to the file during the year from a computer comparison of the NCI and CAS registry files. There are now approximately 85,000 CAS registry

numbers listed as synonyms for the 186,000 compounds on the file. The CAS registry number is assigned during the processing of all chemicals mentioned in articles abstracted by CAS since 1965 (over 2,200,000 unique substances), and is useful as a link to other files.

D. Microfilming

With all master files having been filmed, efforts continued during the year to correct discrepancies found in the film and to film additions to the file. After the film was checked, the file of CAS-annotated copies of the master file was destroyed, and plans are being made to store the old "rough" chemistry card file at the Federal Records Center since there have been no additions to this file since NSC-151,085. The present file procedure consists of keeping a xerox copy of each master card until filming is complete, and then using the master card and film as the chemistry card reference.

Chemical File Analysis

SRS is periodically asked to perform analyses of the data bases it maintains or builds, in order to provide succinct management tools to DCT investigators.

The Section provided an analysis of active agents by rate of acquisition and an analysis of active plant materials, and cooperated in a preliminary study of categorization of active compounds by their "Family Code."

The Section was also heavily involved in the DR&D project to assign priorities to compounds in the screen. Using statistics available for the master file and active (SAC) file, graphs were drawn showing the relative distribution of nitrogen, oxygen, phosphorus, and sulfur, and the total atom population in the two files.

It was hoped that the differences in distribution of active vs inactive compounds could be used to partition all new input of chemicals to the antitumor screen. Thus, if the active file showed a higher nitrogen content than the master, new nitrogenous input could conceivably be given priority in testing. Analyses were also made of the totals of possible low priority classes and Section personnel cooperated in a study of the molecular weight distribution of the SAC file as another clue to priority assignment.

In addition to the file analysis, several methods to implement priority schemes were investigated, both within DR&D and through discussions with CAS. Methods discussed ranged from detection of analogs of interest (and disinterest) at time of input to a categorization based on the routine analysis of connectivity performed by the computer-edit programs for all new structures input to the NCI master file.

A selection and listing of names for all SAC compounds with a "special testing" code was presented to DEB for the purpose of purging some of these from the SAC list. Booklets of chemical names of all SAC compounds plus those having

DN-2 activity or greater was produced for the DDB. This booklet was used in the evaluation of the DCT synthetic program by its advisory committee.

Chemical-Biological Interaction

Because of its responsibility for maintaining both the chemical and biological files, the Section plays a pivotal role in all studies of structure-activity relationships.

A. Substructure Search

The ability to search all chemicals in the file for selected fragments (such as purine rings) is the most useful tool in structure-activity studies. Search is used in structure-function studies by the Drug Evaluation Committee (DEC) and at Decision Network 2 of the linear array. In addition, it is available as a retrieval tool for interested DCT scientists and, on a more limited basis, to qualified outside requesters. During the year, the Section continued to use the CAS substructure search system located at CAS, and in addition began running selected searches at the NIH Computer Division. An agreement between PRA and the DCRT Data Management Branch allowed the simplification of the procedures for submitting, running, and keeping track of files produced during search, and will allow more routine use in the future.

B. Searches Run

One hundred and five new searches of the master file were run during the year, together with 75 updates of previous searches. These included updates to the 1,4-quinone study, a new folic acid study, and an exhaustive compilation of metals tested by DR&D to allow strict monitoring of a synthesis contract in this area. Forty-five active analog searches were run, primarily for the purpose of aiding the DEC and Decision Network meetings in evaluating the biological data of a compound in light of its structural novelty.

Questions were formulated and coded by SRS personnel and checked at CAS before being searched on the master file there. Output was provided to users as lists of NSC numbers, copies of structures, and biological data where requested.

C. Developments in Search

The primary development in search during the year was the cooperation of Section personnel with the University of Pennsylvania in the development of a search system as part of the new chemotherapy chemical information system. Statistical data was furnished on past searches and the overall search needs of DCT in light of past experience.

D. Structure-Activity Correlation

The Section cooperates in all structure-activity correlations in that it supplies the initial data and data handling methods used in each correlation. Two studies in particular stand out as prototypes of future developments in this area.

1. In the first, three searches for folic acid antagonists were made by computer and the results output as machine-processable records. These records were fed directly into a program developed under Mrs. Rhoda Mancher's direction which (a) retrieved and stored all detailed test data for each compound, (b) summarized the data by tumor system and schedule, and showed MTD and highest T/C, allowing an entire class to be studied in summary before detailed analysis began.

2. All summarized data on a large study of 1500 quinones was input into a machine-readable file to serve as the basis for a publication of a study of these compounds. All data tables needed in this study were derived from this data base as well as various cross-references, such as formula-entry number.

   The capturing of this data in computer-processable form eliminated much proofing and manual manipulation and gave valuable experience toward drastically reducing the time needed for this type of study in the future.

Biological File

A. Data Input

The large amounts of data produced under the increased screening program resulted in increased workloads for personnel responsible for (a) checking control packs and control pack indexes; (b) sorting and routing natural product data, and filing both natural product and synthetic data; (c) researching computer error outputs and correcting the master data files; (d) checking shipping list exceptions.

During the year, Section personnel also cooperated fully with the project officer of the biological data processing contract in the training of a new contractor and in checking on the results of implementing improvements to the system.

B. File Maintenance and Inquiries

The Section continued its service of filing screening data summaries and providing copies of data. There were about 120 inquiries for screening data summaries during 1972. Most of these were filled by direct inquiry to the computer file in the weekly production run; however, a significant proportion were filled by photocopying the library copies.

C. Monitoring of Compounds of Interest

Section personnel continued to prepare input and check output for information used in monitoring active agents and those compounds for which more compound was needed for testing. Lists, such as the Material of Interest (MOI), the Confirmed Active Plants (CPAM), the Selected Agents List (SAL), and the Quantity Not Sufficient (QNS) (available on request), serve as reference tools in evaluating natural products, active synthetics, and agents requiring an additional supply. They are also used as reference documents by screeners and other DCT investigators.

Contracts Monitored

A. Chemical Abstracts Service (PH43-65-1021)

Computer files are maintained at CAS to duplicate-check new input, perform substructure search, and provide name and formula indexes. The files now contain over 186,000 structures and 280,000 names and synonyms, including 80,000 CAS registry numbers.

B. University of Pennsylvania (NIH-71-2187)

The Section primarily gave consultation in the area of substructure search, providing analysis of past questions and user habits. In addition, as an assistant project officer, the Acting Head, SRS, was involved in reading and critically evaluating the design documentation and attending several large consultation meetings with the PRA advisory board on this contract.

SUMMARY REPORT

EXPERIMENTAL THERAPEUTICS

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1972 - June 30, 1973

The Experimental Therapeutics area of the Division of Cancer Treatment, comprised of the Office of the Associate Director, the Laboratory of Toxicology, the Laboratory of Chemical Pharmacology, the Laboratory of Molecular Pharmacology and the Laboratory of Tumor Cell Biology, is primarily engaged in fundamental laboratory research programs which investigate the interactions of antitumor agents and other drugs of interest to cancer chemotherapy with biological systems at the whole body, cellular and molecular levels and which elucidate the physiological and biochemical control mechanisms in normal and neoplastic growth. A better understanding of these complex phenomena are directed toward improving the chemical treatment of human malignancies as a single or combined modality. Another major function of the Experimental Therapeutics area is to direct and administer an extramural program through the contract mechanism which is primarily in direct support of the Division of Cancer Treatment drug development program, either through the execution of protocol studies or the conduct of fundamental laboratory and clinical research efforts. Summary reports of the individual laboratories highlight the research projects and significant findings associated with their respective areas.

During the past year the most significant organizational change to occur was the appointment of Dr. Anthony M. Guarino as Chief, Laboratory of Toxicology. Dr. Guarino is well qualified to assume this important post, having transferred from the Laboratory of Chemical Pharmacology as senior investigator with a broad scientific background in fundamental pharmacological and toxicological research. We wish Tony well in his new role.

SUMMARY REPORT

OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1972 -- June 30, 1973

Studies of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid (CSF) in several species of animals have been undertaken. Intravascular infusion, ventriculocisternal perfusions, and computer modelling have been the methods generally employed.

Our studies of the permeability of the blood-brain barrier indicate that this system is very much like a tight cellular membrane. Lipid-soluble compounds exchange rapidly between blood and brain; whereas only very small lipid-insoluble materials, such as water, move readily between blood and brain. Ventriculocisternal perfusion studies from our laboratory have shown that the efficacy of delivery of drugs via the CSF from the point of injection to the target site in the tissue may be severely limited by the rapid clearance of the drug from the CSF and the diffusion distance from the CSF-tissue interface to the target site. It appears from this work that the administration of drugs by way of the CSF will provide adequate delivery of drugs to CNS regions only if the drugs used are virtually lipid-insoluble and the CNS regions are at or near the surface of the brain or spinal cord. Our theoretical and experimental studies of carotid infusions as a means of increasing the uptake of drugs by the CNS indicate that this method of drug administration will only be effective in very specific circumstances. Experimental support of this analysis is currently being generated.

From all of the preceding work, we are formulating the principles which are involved in the exchange of material between blood, brain, and CSF and the guidelines which should be used in planning route and dosage schedules for maximal CNS uptake and distribution of drugs. To obtain the most uniform and wide spread distribution of drugs to the central nervous tissue, CSF, and meninges, the drug should be introduced into the blood stream; yet for this route of delivery to provide adequate uptake, the drug must be lipid soluble enough to readily cross the capillaries of the brain and spinal cord. Lipid insoluble drugs, unless transported by special processes, enter brain tissue from the blood very slowly and thus, distribute poorly to the CNS following intravascular administration. On the other hand, compounds which do not cross the blood-brain barrier may be effectively delivered to the brain, spinal cord, and meninges by way of the CSF. There are, however, significant problems, such as adequate mixing throughout the CSF compartment and depth of tissue penetration, when this route of administration is used to deliver drug to CNS tissue. Before infusing the drug into the CSF, consideration must be given to the site and manner of infusion, the location of the tissue target site, and the transport properties of the drug.

Our present work on the sites of formation and reabsorption of cerebrospinal fluid suggests that a small portion of the cerebrospinal fluid in normal animals is produced by the periventricular tissue of the brain and that very little CSF is reabosrbed from the ventricular system by the capillaries of the CNS parenchyma. Other basic studies from our laboratory indicates that measurements of brain blood flow based on the tissue uptake of antipyrine are in error by as much as 50% and that for many rapidly exchanging compounds, previous measurements of brain capillary permeability by arterio-venous differences and tissue uptake profiles have been compromised by blood flow limitations.

From our work on comparative physiology and pharmacology, we have found that lower vertebrates offer an interesting array of physiological and anatomical arrangements for studying transport of material between blood, brain, and CSF, and can serve as model systems for certain pathological conditions. The dogfish, Squalus acanthias, lacks two of the customary components of the mammalian CSF reabsorption system, the subarachnoid space and the arachnoid villi; yet the CSF reabsorptive capacity of these fish resembles that of the monkey. These observations demonstrate a marked similarity for CSF reabsorption between the normal dogfish and the pathological condition, non-communicating hydrocephalus, in mammals. Our experiments on the permeability of brain capillaries to organic and inorganic polar materials in fish has shown that a spectrum of permeabilities, ranging from very low (the dogfish and stingray) to high (the nurse shark and hagfish), exists in these lower vertebrates. Published microscopical studies show that correlating differences of capillary structure exist in these animals. These anatomical findings coupled with our physiological observations suggest that the "tightness" of the blood-brain barrier depends upon the "tightness" of the intercellular junctions of the capillary endothelium. A similar correlation between structure and function has been reported from preliminary studies of human brain tumor material. We have also determined from our comparative studies that the size of the brain extracellular space in the dogfish is around 15%.

The Division of Computer Research, in collaboration with the Clinical Center, is trying to develop ways to improve the efficiency of data handling within the Clinical Center. As a part of this effort, we are attempting to make the computer scan the physicians' orders and check them for completeness and logical consistency. At present a simple method of analyzing physicians' orders has been developed and is currently being tested for possible use in the Clinical Center.

Serial No. NCI-3500
1. Office of the Associate Scientific Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1973

Project Title: The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

Previous Serial Number: Same

Principal Investigator: Joseph D. Fenstermacher

Other Investigators: Ronald Blasberg, Walter Eckman, Warren Lux, Alan Cowles, Clifford Patlak, David P. Rall, Leon Sokoloff, and Robert Phair

Cooperating Units: Biometrics Branch, NIMH; Office of the Director, NIEHS; Laboratory of Cerebral Metabolism, NIMH; Laboratory of Theoretical Biology, NCI; DCRT

Man Years:

Total:	6.0
Professional:	3.5
Other:	2.5

Project Description

Objectives:

The objectives of this project are to quantify the transport of various materials of physiological and pharmacological importance between blood, brain, and cerebrospinal fluid (CSF) and to study the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. amino acids, within the blood-brain-CSF system. It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to develop methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The method of ventriculocisternal perfusion of various radioactive materials and subsequent brain tissue sampling has been utilized in monkeys and dogs for measurements of brain capillary and cellular permeability and CSF production and reabsorption. A constant pressure flow system has been employed for studies of the CSF absorption capacity in normal and hydrocephalic dogs and monkeys. An experimental system employing simultaneous intracarotid and intravenous infusions has been devised and used for assessing the effectiveness

of these routes of drug administration. Studies of brain and CSF uptake and distribution of intravenously administered, radioactively labeled physiological and pharmacological materials have been undertaken in dogs, monkeys, and elasmobranchs. Finally, a technique for changing or "clamping" the blood-CSF electrical potential is being developed for our studies on the effects of electrical gradients on the flux of inorganic ions between blood, brain, and CSF.

Major Findings:

With our ventriculocisternal perfusion studies we have continued our measurements of the permeability of brain capillaries to various materials and begun to examine the formation, flow, and reabsorption of fluid within the brain parenchyma. We have determined that the brain capillaries of the rhesus monkey are virtually impermeable to a spectrum of extracellular molecules ranging in size from thiocyanate to inulin. The permeability of these capillaries to small polar compounds, such as urea and ethylene glycol, is much less in monkeys than in dogs. Furthermore, our antipyrine studies in monkeys shows that the blood to brain exchange of this material is much lower than has been assumed in brain blood flow studies which use antipyrine as a flow limited marker. Our preliminary results on the formation, flow, and absorption of fluid by the brain suggest that a small portion of the cerebrospinal fluid may be formed by brain tissue and flow into the ventricular and subarachnoid spaces.

Our work on the distribution of antitumor agents into brain tissue during ventriculocisternal perfusion has strong implications about the efficacy of intrathecal drug injections and infusions. Lipid soluble drugs, such as thiotepa and BCNU, do not penetrate very deeply into brain tissue (<1.0 mm) because of their rapid exchange across brain capillaries and removal by the circulatory system. Also for these drugs, their rapid loss from CSF to tissue to blood acts to quickly clear the CSF of drug and minimize the mixing or distribution of the material throughout the CSF system. In contrast methotrexate and hydroxy-urea, two polar compounds, did not leak appreciably across the brain capillaries. Their depths of penetration are limited by the bulk delivery of entrained drug by the CSF, their respective diffusibilities through the brain, and the duration of perfusion or infusion. Less clearance from CSF by tissue and blood uptake occurs with these drugs, and thus, better distribution through the CSF system is obtained than with thiotepa and BCNU. Another polar antitumor agent, cytosine arabinoside, was rapidly cleared from the CSF. This was a result of extensive cellular uptake of the material within the tissue adjacent to the CSF, and not transcapillary exchange. The work suggests several guidelines for improving intrathecal drug delivery: 1) that the intrathecal administration of lipid soluble drugs would not lead to extensive distribution throughout the CSF or to significant accumulation in brain tissue and would be of little value in brain tumor chemotherapy: 2) that the intrathecal administration of certain polar drugs may provide adequate amounts of drug to meningeal and nervous tissue sites near the CSF but probably not to deep (>3-4 mm) tissue areas; and 3) that rapid CSF clearance may occur and limit the distribution of certain polar drugs to tissue sites which are

some distance removed from the point of intrathecal administration.

Our computer modeling of intracarotid infusions shows, theoretically, that this route of drug delivery does not greatly enhance the distribution or accumulation of most drugs within the CNS nor does it minimize peripheral drug delivery and systemic toxicity. From this analysis we have tentatively concluded that intracarotid infusions are superior to intravascular infusions only for a drug which is quickly and tightly bound to active sites within central nervous tissue, or is rapidly metabolized within the tissue to a polar, active form which is retained by the tissue, or is exceedingly rapid in its cytotoxic action. Our experimental studies with model compounds appear to be collaborating these conclusions.

Mathematical drug distribution studies with our physiological, whole body computer model have been made. Using this approach variations in routes, duration of infusion, metabolic half-life, excretion, and other physiological and pharmacological parameters have been examined with particular emphasis on brain tissue, bone marrow, and digestive tract delivery patterns. From this work we have concluded that one can indeed successfully determine general distribution patterns for any given model compound for an endless variety of sets of injection or infusion parameters; however, without adequate information on the drug's mechanism and kinetics of action, optimal administration schedules can not be devised. When such information is available, this drug distribution model can be usefully employed to aid in designing chemotherapy protocols.

An experimental and computer analysis of the methods used to measure cerebral blood flow has clearly demonstrated that values obtained with antipyrine are from 20 to 50% too low. This is a result of the inadequacy of the assumption that brain capillary permeability is very high for antipyrine. More lipid soluble, and thus more permeable, materials such as trifluoroiodomethane give more reliable estimates of brain blood flow than does antipyrine. As an ancillary part of this project we also determined, both experimentally and theoretically, that measurements of brain capillary permeability by arterio-venous differences and net brain uptake are in error for compounds with intermediate (e.g. antipyrine) to high (e.g. thiopental) capillary exchange rates. This error is the result of the assumption of tissue uptake being virtually completely limited by blood-brain barrier permeability. On the basis of this, we have suggested that accurate measurements of brain capillary permeability can be made with these techniques if cerebral blood flow measurements and considerations are included in the experimental procedure and analysis.

We have continued our studies of the comparative physiology and pharmacology of blood-brain-CSF transport. Using a technique of intracerebellar ventricle injection, the first physiological estimates of the size of the brain extracellular space in sharks were made. Based on the inulin, sucrose, and mannitol distribution spaces, an extracellular space of 15-18% was determined for two regions - the medulla and the cerebrum - of the brain. This value is from three to five fold larger than that estimated from electron micrographs

and suggests that a large functional brain extracellular space exists in these animals. The cerebrospinal fluid absorption capacity was measured in the spiny dogfish, an animal which lacks two of the anatomical structures - a subarachnoid space and arachnoid villi - involved in the reabsorption of CSF in higher vertebrates. Despite these marked anatomical differences, the CSF absorption capacity of the dogfish is similar to that of dogs and monkeys, suggesting that other routes of absorption may operate just as effectively for CSF removal as those present in higher vertebrates.

The Division of Computer Research and the Clinical Center are seeking a computerized method for keeping physicians' orders, charting, diagnostic tests, etc. in order and checking for logical consistency. As a part of this project, we have devised a simple way to make the computer scan the physicians' orders and check them for completeness, accuracy, and soundness. This technique is currently being tested for possible use in the Clinical Center.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the brain, spinal cord, and meninges. Anti-tumor agents can be delivered to these tissues by the vascular and/or the cerebrospinal fluid systems; however, each of these two systems appears to have certain limitations as a means of effective drug delivery. Basic information concerning the transport physiology and movement of material into normal and neoplastic CNS and meningeal tissue from the blood and from the CSF is needed for the design and development of effective brain tumor chemotherapy protocols. Such information about blood-brain-CSF transport coupled with a knowledge of the physiochemical and pharmacological properties of specific antitumor drugs will enable the chemotherapist to choose the best route, manner, and timing for effective drug delivery to CNS and meningeal lesions.

Basic knowledge of the transport of ions, metabolites, and other materials between blood, brain and CSF is essential to the understanding of the activities and functions of the central nervous system. Since our work is fundamental in nature, it is relevant to work being carried out by many other groups of bio-medical researchers, and important for an understanding of both normal and pathological brain and spinal cord processes. The type of work performed in our laboratory on this subject is, thus, meaningful and significant to the neurologist, the neuroncologist, the neurosurgeon, and the neuropathologist.

Proposed Course:

1. Continue our studies of the transport of various physiological and pharmacological materials between blood, brain, and CSF and more precisely quantitate the permeability of brain capillary and cellular membranes.

2. Investigate further the formation, flow, and absorption of cerebrospinal fluid by and through CNS tissue.

3. Experimentally examine the brain uptake of drugs when infused intracarotidly and evaluate the effectiveness of this route of administration of chemotherapeutic agents.

4. Continue the use of our computer model of drug distribution and generate suggestions on routes and schedules for more effective drug delivery to the CNS with lower systemic toxicity problems.

5. Proceed with our studies of the mechanism, sites, and capacity for CSF absorption in normal, hyper- and hypo-vitaminic A animals.

6. Examine the effects of perturbations of the blood to CSF electrical gradients upon the distribution of ions and the formation of CSF.

7. Begin an examination of the effects of various membrane active agents, such as vitamin A and some heterocyclic antibiotics, on brain capillary and cellular permeability.

8. Investigate further the comparative physiology and pharmacology of material transport between blood, brain, and CSF in lower vertebrate since these animals offer certain anatomical and physiological advantages for the study of material and fluid exchange and can serve as natural models of various neuropathological situations.

Publications:

1. Fenstermacher, J. D.: Ventriculocisternal perfusion as a technique of studying transport and metabolism within the brain. In Marks, N. and Rodnight, R. (eds.): Methods in Neurochemistry. Plenum Publishing Corp., New York, 1972, pp. 165-178.

2. Fenstermacher, J. D., Dvorchick, B. H., and Maren, T. H.: Volume changes of cerebrospinal fluid in Squalus acanthias during carbonic anhydrase inhibition. Comp. Biochem. Physiol. 44A: 229-234, 1973.

3. Cowles, A. L., and Fenstermacher, J. D.: Theoretical considerations in the chemotherapy of brain tumors. In Sartorelli, A. G. and Johns, D.G. (eds.): Handbuch der Experimentellen Pharmakologie (Antineoplastic and Immunosuppressive Agents). Berlin, Springer-Verlag (in press).

Serial No. NCI-3516
1. Office of Assoc. Sci. Dir. for Exp. Ther.
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents

Previous Serial Number: Same

Principal Investigator: J.A.R. Mead

Other Investigators: A. E. Auletta, C. T. Hardesty, L. C. Mishra and R. Tomchick, Microbiological Associates, Inc.; A. Goldin, H. B. Wood, Jr., J. Driscoll, J. M. Venditti, A. W. Schrecker and D. G. Johns, NCI; W. R. Jondorf, University of Glasgow.

Cooperating Units: None

Man Years:

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives:

To investigate the biochemical pharmacology and biochemical toxicology of antitumor agents in experimental preclinical systems to better define the parameters which influence the chemotherapeutic activity of new or established agents.

Methods Employed:

Standard laboratory procedures were employed.

Major Findings:

The studies in this project can be categorized under the following headings:

A. Folic Acid Antagonists
B. Homofolate Derivatives
C. In Vivo Assay of Dihydrofolate Reductase
D. Ellipticine and its Derivatives
E. Cyclocytidine

F. Antiparasitic Compounds

A. Folic Acid Antagonists

Further work on a series of methotrexate derivatives has shown that although there is a considerable variation in the susceptibility of individual members to oxidation by rabbit and mouse liver aldehyde oxidase it is difficult to make a good correlation between dose level and therapeutic effectiveness. Further comparative work along these lines is needed before it will be possible, if at all, to select an agent with properties sufficiently different from methotrexate to justify further developmental work. One agent of particular interest in this series was a methotrexate analog in which the p-aminobenzoate moiety has been replaced by a partially reduced quinoline moiety in such a way as to preserve the conformation of the N_{10} relative to the pteridine moiety. This agent showed antileukemic activity at least as good as methotrexate and was also equally potent as an inhibitor of dihydrofolate reductase. One apparent discrepancy between reductase inhibition and biological effect was observed with the compound we have designated as meta-methotrexate. This agent was quite a potent inhibitor of mouse liver dihydrofolate reductase in vitro but was devoid of biological activity against advanced subcutaneous L1210. The basis for this discrepancy appears to be a permeability barrier because injection of a dose of 10 mg/kg into mice did not cause any inhibition of liver dihydrofolate reductase 2 hours later. A comparable dose and time interval for methotrexate resulted in complete inhibition of the enzyme, assayed in vitro. Further work will be conducted with this group of compounds to see whether there exists some property which identifies an agent as a uniquely active compound worthy of additional evaluation as a potential clinical agent.

B. Homofolate Derivatives

The development of 5-methyl-d,l-tetrahydrohomofolate as a potentially useful clinical agent is proceeding well. Several more observations have been made which suggest that this agent will be well worth evaluating in the clinic providing it passes its preclinical toxicologic hurdle. It should be emphasized strongly that it represents a class of antifolate completely distinct from methotrexate, since it has a marked inhibitory effect on induced methotrexate-resistant leukemia L1210 and is devoid of any inhibitory effect on mouse liver dihydrofolate reductase at concentrations as high as 10^{-4}M. During the past year the agent has continued to show a greater effect against antifolate-resistant variants of L1210 than against the parent tumor and perhaps more significantly this greater activity is not dependent on the presence of elevated levels of dihydrofolate reductase. The drug produces significant increases in survival time in leukemic mice when given orally or when the tumor is inoculated intracerebrally although the dose levels are somewhat higher than those giving maximal effects after parenteral administration.

From biochemical considerations it was reasoned that the agent might be interfering with the biosynthesis of methionine from homocysteine. However,

in a series of experiments it was found that the biological effect of the 5-methyltetrahydrohomofolate, while readily reversed by folate, citrovorum factor and 5-methyltetrahydrofolate was not reversed by methionine, homocysteine or S-adenosylmethionine. Whether or not vitamin B_{12} would be needed in conjunction with the latter 3 compounds is unknown but induction of a methionine deficiency by 5-methyltetrahydrohomofolate is apparently not its primary mode of action. These experiments do indicate an additional difference between 5-methyltetrahydrohomofolate and methotrexate namely, folate does not reverse the biological effect of methotrexate on chronic administration whereas it was just as effective as citrovorum factor in reversing the antileukemic effect of 5-methyltetrahydrohomofolate. This is probably another indication of the lack of effect of 5-methyltetrahydrohomofolate on dihydrofolate reductase.

An attempt to get a synergistic effect between methotrexate and 5-methyltetrahydrohomofolate against leukemia L1210 did not materialize. When given concomitantly the effect of the combination was less than methotrexate given alone, suggesting interference of some kind, possibly cell uptake. When the 5-methyltetrahydrohomofolate was given after methotrexate there was increased toxicity but no gain in antileukemic effectiveness.

C. In Vivo Assay of Dihydrofolate Reductase

The method described in last years report has been employed to study dose-effect relationships in vivo with methotrexate as the inhibiting agent. Of particular interest was the extension of the study to tissues in addition to liver and gut and it was found that whereas the equilibrium position for these tissues was greatly in favor of tetrahydrofolate in untreated animals, in other tissues such as tumor and spleen the ratio of tetrahydrofolate to dihydrofolate was only about 2:1 rather than the 50:1 ratio seen in liver. Examination of the effect of various doses of methotrexate on this ratio revealed that in every case the ratio shifted towards dihydrofolate but in no case was the formation of tetrahydrofolate completely shut off. In the face of this observation it is apparent that some revision in our current thinking about the mechanism of action of antifolates may be appropriate since methotrexate is not able to reduce the amount of tetrahydrofolate formed in vivo significantly.

In a study in which the relative inhibition of the enzyme by methotrexate was determined, the activity of an antifolate-resistant variant of L1210 with elevated enzyme levels needed almost 100 times as much drug to reverse the ratio of tetrahydrofolate to dihydrofolate. Since a similar increase in dose level would lead to massive toxicity on a daily treatment schedule it is apparent that the association of the elevated enzyme level with resistance to methotrexate may well be a cause-effect relationship!

D. Ellipticine and its Derivatives

Further work on the distribution of radio-labeled ellipticine has confirmed the presence in mouse feces of several metabolites of ellipticine which have

fluorescent characteristics clearly distinct from the parent drug. Identification of these materials and their synthesis would be highly desirable especially to see whether some of the derivatives lack the propensity to cause hemolysis exhibited by ellipticine. In a related study the effect of Udenfriend's hydroxylating mixture on ellipticine was examined to see whether compounds similar to the metabolites were formed which would suggest, of course, that hydroxylation was a metabolic pathway of some importance. No products similar to the metabolites were found but only trace amounts of drug-derived radioactivity were seen separating from ellipticine on chromatograms. Parallel studies with mouse liver microsomes are planned with and without inhibitors of microsomal enzyme metabolism present.

E. Cyclocytidine

The major question to be resolved with this agent, as far as its biochemical pharmacology is concerned, is whether or not the antileukemic activity is solely due to its conversion to ara-C or whether it may have properties peculiar to itself. So far, all attempts to identify phosphorylated products of cyclocytidine have failed but the technology is not available at present which could guarantee the preservation of cyclocytidine nucleotides in the reaction mixtures without their rapid conversion to ara-C nucleotides. The cyclocytidine-resistant tumor we developed is devoid of phosphorylating activity for cyclocytidine and ara-C.

In an attempt to see whether the cyclocytidine-resistant tumor has properties which would permit us to discriminate it from an ara-C resistant tumor dose-response curves were run for several agents against the two tumors. To date both tumors have shown a similar pattern of response to about 20 agents. This does not, of course, provide unequivocal evidence that is solely an ara-C resistance phenomenon but does suggest that the kinase which is deleted with the onset of resistance is the same for both drugs namely, deoxycytidine kinase. Both resistant-tumors showed an enhanced response to 3-azauridine confirming and extending the earlier observation for an ara-C resistant line at Southern Research Institute.

F. Antiparasitic Compounds

As part of a continuing interest in the antitumor potential of compounds used in the treatment of parasitic diseases a metabolite of hycanthone, desethylhycanthone, mirasane and hydroxymethylmirasane were each administered orally to mice bearing leukemia L1210. No antileukemic effect was seen in any case suggesting that whereas the parasitic conditions might respond under these circumstances the tumor growth was not impeded.

Significance to Biomedical Research and the Program of the Institute:

The acquisition of knowledge pertaining to the physiological distribution and metabolic transformations of antitumor agents is a very important objective of the drug development program of Division of Cancer Treatment, NCI. This project is directed towards the goal of acquiring this information and also

to undertake more fundamental studies to elucidate the relationship, if any, of physiological distribution to chemotherapeutic activity and/or toxicity. This program is an integral part of the scientific endeavor under the aegis of the Experimental Therapeutics Program of the Division of Cancer Treatment.

Proposed Course of Project:

The project will continue to follow a course aimed towards the acquisition of relevant pharmacological, toxicological and biochemical data for input into the Linear Array for drug development. Concurrently, phenomena of a basic nature will be investigated inasmuch as they are related to the physiological effects, in animals, of experimental antitumor agents.

Honors and Awards: None

Publications:

Hardesty, C.T., Chaney, N.A., and Mead, J.A.R.: The effect of route of administration on the distribution of ellipticine in mice. Cancer Res. 32: 1884-1889, 1972.

Hardesty, C.T., Chaney, N.A., Waravdekar, V.S., and Mead, J.A.R.: Studies on the disposition of the antitumor agent, sangivamycin, in mice. Cancer Res. (in press).

Mead, J.A.R.: Rational design of cytotoxic agents: Folic acid antagonists. In Sartorelli, A.C. and Johns, D.G. (eds.): Antineoplastic and Immunosuppressive Agents. New York, N.Y., Springer-Verlag (in press).

SUMMARY REPORT

LABORATORY OF CHEMICAL PHARMACOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1972 - June 30, 1973

The efforts in the Laboratory of Chemical Pharmacology are concerned with the pharmacological properties of potential and established antitumor agents. This includes the disposition, mechanism of action, structure-activity relationships, rational design and dose-response relationships of antitumor drugs and other model foreign compounds in laboratory animals and man. This requires in many instances the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition is not fully understood. Of special concern in disposition studies is the establishment of rates and rate constants which govern the passage of drugs across membranes from one body compartment to another, the assessment of drug biotransformation or binding to cellular constituents or macromolecules, and the determination of the rate of elimination of the drug and its metabolites. When this information is considered together with a drug's chemical and physical properties as well as its route and schedule of administration, predictions can be made concerning the effective concentration of a drug or its metabolites in tumor and susceptible normal tissue - the receptor sites. These studies are particularly important since one of the major factors contributing to the lack of adequate drug response of the host is the failure of the drug to reach the target site in sufficient concentration to eradicate the tumor cells. The knowledge gained from such studies when considered together with biochemical, toxicologic, and cytokinetic data will hopefully permit the design of drug dose-levels and schedules by the clinician for more effective therapeutic use of antineoplastic agents in human malignancies.

In addition, another major effort of the laboratory is to determine, develop and evaluate specific biochemical materials which are products of tumor cells which can be used for diagnostic purposes and to assess the antitumor effects of various therapeutic modalities.

During the past year a number of research projects were continued and several new studies were initiated. Some of the experimental findings are high-lighted below.

The folic acid antagonists continue to be studied in this laboratory and studies this past year reveal that by application of model predictions and measurements of plasma methotrexate in mice, the toxicity of a single dose of methotrexate was found to be due to the drugs persistence in the plasma at a very low concentration for a very long time. The critically effective concentration appears to be about 1×10^{-8}M and the critically effective time appears to be exposure to this concentration for longer

than 60 hours in order for lethality to result. Such toxicity could be prevented by removing the micro-infusion cells as late as 60 hours or in the case of single lethal doses by administering citrovorum factor as late as 48 hours after the initial dose of methotrexate.

Combination of methotrexate and vincristine indicate that additive effects on DNA synthesis occur in the small intestine, bone marrow and tumor, but the greatest effect appears to be in the tumor. However, these are preliminary findings and much more investigation is required.

The uptake patterns of MTX (2μM) were examined in myeloblasts and lymphoblasts in vitro from ALL and AML patients. No definite difference was apparent between the cell types in the 16 patients studied suggesting that sensitivity differences between the ALL and AML patient to MTX cannot be ascribed to variation of transport. In human leukemia cells, 60% enhancement of MTX transport was demonstrated for vincristine (10^{-4}M) whereas 25-30% inhibition was shown for hydrocortisone (10^{-4}M to 10^{-5}M) and for cephalothin at 25 μg/ml.

A series of lipid soluble dialkyl esters of methotrexate has been prepared, and the pharmacological activity of these compounds has been assessed in vivo and in vitro. The dialkyl esters were effective inhibitors of dihydrofolate reductase from mouse L1210 leukemia cells; their inhibitory activity, however, was less than that of the non-esterified parent compound, MTX. Within the ester series, enzyme inhibitory activity increased with ester chain-length up to a maximum with the 4- and 5-carbon esters; further increases in chain-length were accompanied by a decrease in inhibitory activity. In contrast to the results obtained in the dihydrofolate reductase inhibition assay, the antitumor activity of the esters in vivo, in terms of extension of life-span, was equal to that of MTX when tested against both the intracerebrally and intraperitoneally implanted tumor. The maximum effective dose of the esters was also the same as that of the parent compound (1.5 mg/kg/day IP) except for the diethyl ester, which was maximally effective at a dose of 4 mg/kg/day. In the mouse and rat, the dialkyl esters are very rapidly hydrolyzed in vivo to yield the parent compound, methotrexate, and their pharmacological properties therefore resemble those of the latter drug. In the dog and monkey, however, these compounds persist for several hours in the esterified form. Further studies of these compounds will be undertaken in the monkey in order to ascertain the potential usefulness of these compounds for the treatment of meningeal leukemia and CNS tumors in man.

Carboxypeptidase G_1 (CPD G_1) a folate cleaving enzyme from Pseudomonas stutzeri has been further purified and studies in mice have shown that it has no effect on DNA synthesis in bone marrow and GI epithelium while at the same time it inhibits both UdR and TdR incorporation in L1210 cells. In addition, CPD G_1 is able to cleave circulating levels of methotrexate and to "rescue" animals from lethal methotrexate if given 24 hours after the antifolate. This therefore represents a new approach to prevention of the toxic effects of anticancer drugs.

Kinetic studies with 5-FU were undertaken in mice bearing P1534 a sensitive tumor or Ehrlich ascites, a resistant tumor. A dose dependent inhibition of UdR incorporation into DNA in both normal and tumor tissue was found. The duration of this inhibition was greater in the sensitive tumor then in the resistant tumor. Normal tissues showed an increased sensitivity to second doses of 5-FU during the recovery phase. Exploitable kinetic differences were found in the recovery of normal and tumor tissue so that repeat doses of 5-FU could be given with maximum effectiveness.

Studies were undertaken on the enzyme cytidine deaminase in AML cells since previous studies had shown that increased levels of this enzyme may be induced by cytosine arabinoside in patients with acute myelocytic leukemia. A thirty-fold purification of the enzyme was accomplished and several compounds were found to be substrates or inhibitors for the enzyme. In particular 5-azacytidine was found to be a substrate for this enzyme suggesting the possibility of clinical cross resistance to cytosine arabinoside on the basis of increased deaminase activity. In addition, cyclocytidine was found to be neither a substrate nor an inhibitor of the enzyme.

The nitroreductase activity of the enzyme hepatic aldehyde oxidase was further studied. This enzyme was found to be capable of forming the proximate carcinogens from several nitro-substituted N-heterocyclic compounds. Further information is necessary to ascertain the potential relevance of this nitro group reduction to the etiology of neoplastic disease.

Several potential antitumor agents were discovered by rational approaches. Hydroxyguanidine, a compound which combines the imino group of guanidine, an antiviral agent, with the hydroxyamino group of hydroxyurea, an antitumor agent, was found to have antitumor activity in vitro and in vivo against several experimental tumors and antiviral activity against Moloney sarcoma virus. This is a prototype of agents with dual chemotherapeutic activity and suggests that agents may be designed which have cytotoxic effects against the tumor cell and antiviral effects against oncogenic viruses.

Hycanthone was found to have inhibitory effects against various types of rapidly proliferating tissue including tumor cells in vitro and in vivo, PHA stimulated lymphocytes and the embryo. The antitumor activity of hycanthone occurred in several tumors including leukemias L1210, P388, mast cell tumor P815 and Walker 256 carcinosarcoma. Mechanism studies showed that hycanthone inhibited both DNA and RNA but not protein synthesis. Furthermore, hycanthone was found to be a potent inhibitor of the enzyme aldehyde oxidase. These studies suggest that hycanthone may have promise as an antitumor agent, either alone or in combination with antitumor agents known to be inactivated by aldehyde oxidase.

The 3 geometric isomers of DDD were studied utilizing ACTH-induced steroid production, histology and ultrastructure of the dog adrenal cortex. Results indicated that m,p'-DDD is more potent than p,p'-DDD against all three parameters and suggests that this compound may be more useful than o,p'-DDD as an antitumor agent.

Disposition studies were undertaken with several new antitumor agents and potential antitumor agents. 2,6-Dimethyl-4-benzyl-4-demethylrifampicin (DMB, NSC-143414) an inhibitor of reverse transcriptase was found to be orally absorbed and slowly eliminated by several animal species. High tissue/plasma ratios were found in the liver, pancreas and adrenals. Chromatographic studies thus far indicate that the parent(hydroquinone) and quinone forms of the drug are eliminated. Further studies with this compound and with other viral RNA-directed DNA polymerase inhibitors will be carried out.

The disposition of ^{14}C-guanazole has been examined in the mouse, rat and dog. Guanazole was rapidly excreted in the urine of mice and rats after parenteral administration with 90% of the dose found in mouse urine two to three hours following parenteral administration. Excretion of guanazole by the anesthetized dog was somewhat slower than that by the mouse or rat with 80% appearing in the urine within 6 hours. No evidence was found for the metabolism of guanazole and apparently this antitumor agent is excreted unchanged in these three species. The rapid excretion of guanazole offers an explanation for its greater antitumor activity when given frequently rather than on a once a day schedule. Studies of ^{14}C-guanazole in man revealed similar findings to the above in that 60% of the radioactivity was found at the end of 24 hours infusion and 91% recovered after 80 hours. Again chromatographic studies revealed that the majority, if not all the radioactivity found, was unchanged guanazole.

The availability of a large breeding and experimental monkey colony (600 animals representing 5 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, and biochemical studies in subhuman primates. Thus far tumors have been induced in non-human primates by N-nitrosodiethylamine (DENA), 1-nitrosopiperidine, procarbazine, aflatoxin B_1 and cycasin. Alpha-feto-protein (AFP) can be detected by double diffusion techniques in monkeys receiving DENA 6-8 months prior to histological evidence of tumor, this allows prediction of which monkeys will develop tumor and is a premalignant model useful for immunotherapy. In addition, AFP has been detected in vitro from cells from biopsy derived liver tumors. This provides a model system for calculating total body synthesis of AFP and the average cellular synthetic rate and thereby estimate the total number of liver tumor cells in the body.

Methods have been developed to evaluate whether or not specific materials are elevated in the blood or urine of cancer patients. Initially, patients with advanced disease have been entered into these studies. Preliminary results indicated that specific tRNA degradation products are increased in the urine of some patients with cancer. Further studies are necessary to determine the extent of these elevations and the types of tumors associated with increased excretion. Polyamines appear to be slightly elevated in certain cancer patients urine. Further studies regarding methodology are necessary before final conclusions can be reached. To date studies by radioimmunoassays for methylated nucleic acid bases in serum have been disappointing. Sufficient sensitivity as yet has not been reached in order to detect these compounds by this technique.

In addition to the basic laboratory research effort, each senior investigator shares the responsibility for the administration and supervision of one or more contracts with outside research institutions. The contracts are primarily concerned with development of analytical-biochemical methodology, the study of pharmacological disposition of antitumor drugs and their metabolites in man, laboratory animals, and in in vitro predictive systems, the development of pharmacokinetic mathematical models and the development of biological markers for detection of cancer and assessment of cancer treatment. Approximately 12 new agents are studied each year, each of which reflect the interest of the Division of Cancer Treatment Program area for developing antitumor drugs for the clinic. It is expected that the number of agents to be studied through the contract mechanism will increase with the expanding program on the national level.

Serial No. NCI-3502
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology and Drug Metabolism Sections
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Folic Acid Antagonists

Previous Serial Number: Same

Principal Investigators: David G. Johns, Daniel S. Zaharko, Werner Bleyer, and Vincent T. Oliverio

Other Investigators: Robert L. Dedrick, Robert Lutz, and Vernon Wong

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS, and Clinical Branch, National Eye Institute

Man Years:

Total:	4
Professional:	2
Other:	2

Project Description:

Objectives:

I. Pharmacokinetics and Effect

The continued use of pharmacokinetics and computer simulations of drug kinetics in order to gain further insight into the limitations and future potential of folic acid antagonists. To utilize these methods to study drug kinetics and effects in animals bearing solid tumors.

We have studied the inhibition of DNA synthesis in small intestine, bone marrow, solid tumor by following the incorporation of tritiated deoxyuridine into DNA of Lewis Lung tumor-bearing mice. These studies have been conducted using single injections of methotrexate alone, methotrexate in combination with vincristine, and methotrexate given by constant infusion.

In order to achieve this latter goal of constant infusion into mice, we have designed a micro-infusion cell which when implanted subcutaneously into mice releases methotrexate at a constant rate such that it simulates plasma kinetics similar to what would be obtained by a constant intravenous infusion.

II. Studies Using High-Pressure Liquid Chromatography

This relatively new technique is being investigated as a means of improving drug analysis. It provides a rapid means of separating drugs from their metabolites and also of identifying specific biologically endogenous materials such as nucleotides and nucleosides. Objectives will be to develop systems for separating various drug components. The system will also be used to investigate the effect of various chemotherapeutic agents on endogenous body pools of a variety of markers depending on the sensitivity of the detector system and the capabilities of separation techniques.

III. Transport of Methotrexate and Metabolites across Mammalian Cell Membranes

Studies are continuing to evaluate the effects of certain commonly administered "drugs" on methotrexate (MTX) in L1210 murine leukemia cells and human leukemia cells in vitro as a model for studying potential drug interactions in man.

IV. Lipid-soluble Derivatives of Methotrexate

When administered intrathecally, methotrexate is of value in the therapy of meningeal leukemia, a frequently observed complication of acute leukemia of childhood. When administered by the more convenient oral or intravenous routes however, the drug does not readily pass the blood-brain barrier. It is likely that the poor transport of methotrexate from the systemic circulation into the cerebrospinal fluid is a consequence of its physicochemical properties, since its lipid solubility is extremely low and the carboxylic acid groups of the glutamate moiety of the molecule are ionized at physiological pH.

V. Pharmacokinetics of Intrathecal Chemotherapy for Meningeal Leukemia

During the last decade, an increasing number of children have apparently been cured of acute leukemia. The primary reason for this progress is the addition of central nervous system therapy to all patients whether or not they have evidence for meningeal leukemia. However, intrathecal chemotherapy is only partially effective and frequently associated with serious neurotoxicity (10-30% of all patients). These limitations are attributable, in part, to the lack of pharmacokinetic data and the empirical use of intrathecal chemotherapy. These considerations have prompted evaluation of cerebrospinal fluid and serum drug concentrations in all patients receiving intrathecal MTX. MTX was chosen for study because it is the most widely used intrathecal chemotherapeutic agent.

Methods Employed:

Chromatographically pure MTX-3',5'-T was employed throughout. Drugs were administered by the intravenous, intraperitoneal, or intraduodenal routes. Standard surgical procedures and specimen collection techniques were employed throughout. In other experiments, mouse faecal contents were incubated with tritium-labeled MTX and the metabolites recovered. MTX metabolites were characterized by their chromatographic behavior in several systems and by their ultraviolet and infra-red absorption spectra.

The methodology employed for short-term *in vitro* uptake and efflux studies of MTX in L1210 leukemic cells and human leukemic blast cells was adapted from the procedures of Goldman *et al*. (*J. Biol. Chem*. 243: 5007, 1968). Survival studies were done using the transplantable, ascitic L1210 tumor in CSF_1 mice. In addition, ascites cells from these mice were used for *in vitro* MTX uptake studies and for experiments to determine the number of ascitic tumor cells per mouse.

Major Findings:

I. Methotrexate Kinetics and Toxicity

By application of model predictions and measurements of plasma methotrexate in mice, it has been demonstrated that the toxicity of a single dose of methotrexate is due to the drugs persistence in the plasma at a very low concentration for a very long time. The critically effective concentration appears to be about $1x10^{-8}$M and the critically effective time appears to be exposure to this concentration for longer than 60 hours in order for lethality to result.

The micro-infusion cells are able to maintain such a plasma concentration of MTX and cause lethal toxicity if left in the animals beyond 60 hours.

Such toxicity can be prevented by removing the infusion cells as late as 60 hours or in the case of single lethal doses by administering citrovorum factor as late as 48 hours after the initial dose of methotrexate.

Combination of methotrexate and vincristine indicate that additive effects on DNA synthesis occur in the small intestine, bone marrow and tumor, but the greatest effect appears to be in the tumor. However, these are preliminary findings and much more investigation is required.

Constant infusions of MTX in tumor-bearing mice, that achieve plasma concentrations which are lethally toxic, only inhibit deoxyuridine incorporation into DNA to 30-50% of non-MTX treated controls in bone marrow and small intestine where as in the primary tumor the initial inhibition similar (approximately 30% of control) but recovery appears to occur even during the presence of supposedly toxic methotrexate plasma concentrations. These are preliminary data and further investigations are required to substantiate them and to see whether these phenomenon can be exploited by drug combinations.

II. A system has been developed for separating methotrexate into four components. This separation can be achieved in 20 minutes.

A system has been used that will separate rifampicin and some of its derivatives from one another. The observation was made that rifampicin was broken down to its quinone derivative during elution from the column.

III. The uptake patterns were examined of MTX (2μM) in myeloblasts and lymphoblasts in vitro from ALL and AML patients. No definite difference was apparent between the cell types in the 16 patients studied suggesting that sensitivity differences between the ALL and AML patient to MTX cannot be ascribed to variation of transport.

In human leukemia cells, 60% enhancement of MTX transport was demonstrated for vincristine (10^{-4}M) whereas 25-30% inhibition was shown for hydrocortisone (10^{-4}M to 10^{-5}M) and for cephalothin at 25 μg/ml.

IV. Lipid-soluble Derivatives of Methotrexate

A series of lipid soluble dialkyl esters of methotrexate has been prepared, and the pharmacological activity of these compounds has been assessed in vivo and in vitro. The dialkyl esters were effective inhibitors of dihydrofolate reductase from mouse L1210 leukemia cells; their inhibitory activity, however, was less than that of the non-esterified parent compound, MTX. Within the ester series, enzyme inhibitory activity increased with ester chain-length up to a maximum with the 4- and 5-carbon esters; further increases in chain-length were accompanied by a decrease in inhibitory activity.

In contrast to the results obtained in the dihydrofolate reductase inhibition assay, the antitumor activity of the esters in vivo, in terms of extension of life-span, was equal to that of MTX when tested against both the intracerebrally and intraperitoneally implanted tumor. The maximum effective dose of the esters was also the same as that of the parent compound (1.5 mg/kg/day IP) except for the diethyl ester, which was maximally effective at a dose of 4 mg/kg/day.

In the mouse and rat, the dialkyl esters are very rapidly hydrolyzed in vivo to yeild the parent compound, methotrexate, and their pharmacological properties therefore resemble those of the latter drug. In the dog and monkey, however, these compounds persist for several hours in the esterified form.

As substrates for the drug-metabolizing enzyme aldehyde oxidase, the short-chain esters are highly active, being converted to the corresponding 7-hydroxy derivatives; the Michaelis constants for the hydroxylation reaction are in the range of 0.002-0.005 mM, 10- to 100-fold lower than for previously described substrates for this enzyme. Increases in the ester chain-length are accompanied by increased lipid solubility and decreased substrate activity. With the nonesterified patent compound, methotrexate, the Michaelis constant decreases and oxidation rate increases with decreasing pH throughout the physiological pH range; with the esters, on the other hand, a well-defined maximum in apparent affinity and reaction rate is seen at pH 8. This disparity in behavior for the two types of substrates supports the hypothesis that the substrate activity of the nonesterified parent compound is a property of the undissociated carboxylic acid, rather than the anionic form, and that the apparent enhancement in substrate activity seen with the esters is due to their inability to form the latter.

V. Pharmacokinetics of Intrathecal Methotrexate in Man

The pharmacokinetics of intrathecal (IT) methotrexate (MTX) were defined by data derived from 70 injections in 27 patients. Following IT administration, cerebrospinal (CSF) MTX concentration declines in three phases: 1) rapid distribution of the antifolate within the CSF volume; 2) relatively slow transport of the drug out of the CSF ($t_{\frac{1}{2}}$ = 4-5 hr.) between 3 and 24 hours and 3) a slower efflux component ($t_{\frac{1}{2}}$ = 12-16 hr.) between two and seven days post-injection. The second and third phases are first order over a dosage range of one to 15 mg/M^2. After a single dose of 12 mg/M^2, the CSF level does not fall below 10^{-8}M until 60 hours, and the serum concentration exceeds 10^{-7}M between 3 and 7 hours. Clearance of MTX from the CSF appears to diminish with age and in the presence of overt meningeal leukemia.

To date, every patient with severe neurotoxicity has demonstrated markedly elevated CSF antifolate levels (5 to 100-fold higher than mean levels in nontoxic patients). This observation implys that the neurotoxicity associated with IT MTX therapy can be reduced, and possibly avoided, by frequent monitoring of CSF MTX concentration and appropriate dosage modification.

The results of these studies have been applied to the clinic in a new protocol for all patients with meningeal leukemia. Low doses of MTX are administered intraventricularly (via a subcutaneous reservoir) every 12 hours for three days, during which a minimal blastocidal level is maintained in the CSF. This "concentration x time" therapy is repeated every other week during induction and consolidation, and once monthly thereafter. The first patient to enter this protocol has been in a continuous remission for 148 days, his longest complete remission since diagnosis 21 months ago.

Significance to Bio-medical Research and the Program of the Institute:

Among the active antitumor folic acid antagonists, MTX is the most widely used, and is clinically effective in the treatment of acute lymphocytic leukemia, choriocarcinoma, Burkitt's lymphoma, primary hepatocellular carcinoma, and epidermoid cancer of the head and neck. Because of the great clinical importance of MTX, a complete knowledge of its pharmacology is of practical value. With this established agent it is envisioned that additional information on its distribution and metabolism in a variety of species, and a further understanding of its uptake characteristics into malignant cells alone or in the presence of other drugs, will lead to its improved use and the design and use of newer more effective drugs.

Proposed Course:

I. Methotrexate Kinetics and Toxicity

Further investigations of drug kinetics and effect on solid tumor-bearing mice will be continued. Studiss concerned with drug combinations given in sequences based on observed effects on DNA synthesis and knowledge of drug kinetics will be carried out.

II. To continue along similar investigative lines.

III. Studies of drug-drug interactions of MTX in human peripheral and marrow cells will be continued using in vitro techniques and the L1210 leukemia cell system in vitro and in vivo as a reference.

IV. Lipid-soluble Derivatives of Methotrexate

Studies on the pharmacological activity of lipid-soluble dialkyl esters of methotrexate, and of other lipid-soluble folate antagonists, will be continued in several mammalian species, and toxicity studies will be initiated if warranted.

V. In all forms of metastatic malignancy, prolonged survival has been tempered by an increasing incidence of central nervous system involvement. Concomitantly, the use of IT MTX is rapidly increasing, not only for treatment (e.g. primary brain tumors, choriocarcinoma, lymphoma, but also for prophylaxis (e.g. Ewing's sarcoma). It is imperative that the pharmacokinetics of IT MTX, and of other IT agents be more carefully delineated. The mechanism of neurotoxicity must be established, as well as the role of other factors affecting the pharmacology of the IT chemotherapy.

Publications:

Zager, R. F., Frisby, S. A. and Oliverio, V. T.: The effects of antibiotics and cancer chemotherapeutic agents on the cellular transport and antitumor activity of methotrexate in L1210 leukemia. Cancer Res., in press, 1973.

Oliverio, V. T. and Guarino, A. M.: Abosrption, protein binding, distribution and excretion of drugs. Proc. of the Symposium on Clinical Pharmacology, Jacksonville, Fla., March 11-13, 1973. Biochem. Pharmacol., in press, 1973.

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Johns, D. G., Farquhar, D., Chabner, B. A., Wolpert, M. K. and Adamson, R. H.: Antineoplastic activity of lipid-soluble dialkyl esters of methotrexate. Experientia 29: (6), 1973, in press.

Vogel, C. L., Adamson, R. H., DeVita, V. T., Johns, D. G. and Kyalwazi, S. K.: Preliminary clinical trials of dichloromethotrexate in hepatocellular carcinoma. Cancer Chemother. Rep. 56: 249-258, 1972.

Serial No. NCI-3505
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Potential Carcinogenic Activity and Chronic Toxicity of Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Vincent T. Oliverio

Other Investigators: None

Cooperating Units: Laboratory of Pathology and Laboratory of Toxicology, NCI

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives:

The susceptibility of newborn and adult animals to chemical carcinogens and, particularly, drugs of interest in cancer chemotherapy and antiviral agents and those to which the human population may be exposed is of vital necessity and of particular relevance to the program of NCI. The characterizations and treatment of such tumors is of both theoretical and practical importance. The specific objectives are:

To determine the carcinogenic activity of chemicals to which the human population may be exposed, particularly drugs of interest in cancer chemotherapy, such as alkylating agents, antimetabolites, agents that intercalate with DNA, polymers and various antiviral agents and antibiotics.

To ascertain unusual pharmacologic and toxic properties of various antibiotics and antiviral agents which are used in patients with neoplastic disease.

To characterize and transplant carcinogen-induced tumors as new tools for experimental cancer chemotherapy.

To compare the susceptibility of newborn vs. adult animals to chemical carcinogens.

Methods Employed:

Usual biologic, pharmacologic, and histopathologic techniques.

Major Findings:

Hycanthone is presently under evaluation as a potential carcinogen in CDF_1 mice. Hycanthone is under evaluation because it is widely used in South America and Africa for the treatment of schistosomiasis and over 100,000 people have been exposed to the compound, and because it produces embryotoxic effects in the pregnant mouse and chromosomal aberrations in human PHA-stimulated lymphocytes at high doses. The mice will be autopsied 1 year after dosing ceased (July 1973).

Several interferon inducers including poly I·poly C and tilorone have been evaluated for potential carcinogenic effects. Data thus far suggest that these compounds are not highly carcinogenic in CDF_1 mice.

Studies with poly I·poly C in guinea pigs failed to produce anaphylaxis. Studies of poly I·poly C toxicity in dogs and rhesus monkeys demonstrated that toxicity was dose related and qualitatively similar in both species and included hypoactivity, anorexia, emesis, diarrhea, ataxia and weight loss. Hematological and blood chemistry changes included anemia, elevations of transaminases, lactic dehydrogenase, and alkaline phosphatase, decreased clotting rate and increased prothrombin time.

Procarbazine is under further evaluation as a carcinogen in several species both alone and in combination with other antitumor agents and X-radiation. This is similar to the combination chemotherapy protocol for patients with Hodgkin's disease. Procarbazine has been found to induce tumors in mice, rats and monkeys in our colony and may be suspected of being carcinogenic in humans when administered chronically.

Several antibiotics used in treatment of infections in patients with leukemia and other tumors have been shown to inhibit neuromuscular function. This is of potential importance when patients with various tumors undergo surgical procedures although paralytic episodes have also occurred following administration of certain antibiotics to unanesthetized persons.

Significance to Bio-medical Research and the Program of the Institute:

The possible carcinogenic activity of antitumor agents is not only of clinical importance, but theoretically may help understand the malignant process.

Several drugs used as anticancer agents are known to be carcinogens. Few, however, have exhibited such strong carcinogenic activity as procarbazine, and none elicit such a wide spectrum of tumors in mice and rats. With the clinical use of procarbazine, it is essential to carefully delineate its

carcinogenic potential, not only in rodents, but also in primates both alone and in combination with other anticancer agents.

Unusual pharmacologic and toxic effects of antiviral agents and antibiotics are of major importance to the clinician as these drugs are widely used in patients with neoplastic disease.

The wide exposure of the human population to hycanthone makes it necessary to evaluate the potential carcinogenic activity of this agent and also to evaluate if there is a dose response effect since it is generally administered in humans once at a dose of 3 mg/kg i.m.

Proposed Course:

New antiviral and anticancer agents of clinical interest will be evaluated as potential carcinogens. Tumors that occur from various carcinogens will be evaluated for their applicability to programs for antitumor testing. The pharmacologic effects and toxic properties of antibiotics and antiviral agents used in patients with cancer will continue to be examined.

Publications:

Homan, E. R., Zendzian, R. P., Schott, L. D., Levy, H. B. and Adamson, R. H.: Studies on poly I·C toxicity in experimental Animals. Toxicol. Appl. Pharmacol. 23: 579-588, 1972.

Pittinger, C. and Adamson, R.: Antibiotic blockade of neuromuscular function. Ann. Rev. Pharmacol. 12: 169-184, 1972.

Serial No. NCI-3506
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pharmacology of Alkylating Agents and Other Antitumor Drugs

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson, Susan Sieber Fabro and Richard Cysyk

Other Investigators: Jacqueline Whang-Peng

Cooperating Units: Human Tumor Cell Biology Branch, NCI

Man Years:

Total:	3.5
Professional:	2.5
Other:	1.0

Project Description:

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To investigate the possible embryotoxic effects of anticancer agents and antiviral agents that may be used in malignancy or more generally in non-malignant conditions.

To design newer anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, immunologic, and histopathologic techniques.

Major Findings:

Antitumor Activity of Hydroxyguanidine

Hydroxyguanidine, a compound which combines the imino group of guanidine, an antiviral agent, with the hydroxyamino group of hydroxyurea, an antitumor agent, was found to have antitumor activity in vitro and in vivo against several experimental tumors and antiviral activity against Moloney sarcoma virus. This is a prototype of agents with dual chemotherapeutic activity and suggests that agents may be designed which have cytotoxic effects against the tumor cell and antiviral effects against oncogenic viruses.

Effects of Hycanthone on Rapidly Proliferating Cells

The effects of hycanthone on various types of rapidly proliferating tissue, including tumor cells in vitro and in vivo, PHA stimulated lymphocytes, and embryonic cells, was investigated. Hycanthone was cytotoxic to several tumor cell lines in tissue culture and exerted in vivo antitumor activity against Walker 256 carcinosarcoma, leukemia L1210 and mast cell P815. Human PHA-stimulated lymphocytes incubated in the presence of hycanthone showed a 50% depression in mitotic index, and a marked increase in number of chromosomal aberrations. Hycanthone administered to pregnant mice was not teratogenic, but its embryotoxicity was manifested by an increased incidence of intrauterine death and a decrease in average fetal body weight. Mechanism studies showed that hycanthone inhibited both DNA and RNA, but not protein synthesis in human PHA-stimulated lymphocytes. Furthermore, hycanthone was found to inhibit aldehyde oxidase and this inhibition was greater than that previously found for the N-alkyl-phenothiazines. These studies suggest that hycanthone may have promise as an antitumor agent, either alone or in combination with antitumor agents known to be inactivated by aldehyde oxidase.

Studies with the Antitumor Agent DDD

The effects of 3 geometric isomers of DDD on ACTH-induced steroid production, histology, and ultrastructure of the dog adrenal cortex were examined. When administered i.v., equal doses of all 3 compounds eventually inhibited ACTH-induced steroid production. The order of onset of inhibition was m,p'-DDD>o,p'-DDD>p,p'-DDD with the inhibition reaching 50% of control values at 27 min, 87 min and between 4-18 hr, respectively. Histologic examination of the various zones of the adrenal cortex showed minimal effects, from any of the isomers, on the zona glomerulosa at times when the internal structure and spacial arrangement of the cells of the zona fasciculata and reticularis were markedly disrupted.

Electron micrographs revealed that these DDD isomers exhibited very minimal effects on the mitochondria of the zona glomerulosa. On the other hand, the mitochondria of the zonas fasciculata-reticularis

underwent progressive swelling, dissolution, and eventual rupture following DDD treatment. The order of onset of histologic and ultrastructural effects was m,p'-DDD>o,p'-DDD>p,p'-DDD. However, for each isomer the ultrastructural damage was detected prior to the observation of histologic lesions. Thus, there was a marked temporal correlation between the percentage inhibition of ACTH-induced steroid production, the disruption of normal cellular structure and arrangement in the innermost zones of the adrenal cortex, and the severity of the DDD-induced mitochondria damage in the cells of the zonas fasciculata-reticularis by each of the 3 isomers of DDD.

These results suggest that m,p'-DDD may have a greater antitumor effect then o,p'-DDD and also offers a model system in which the effects of compounds on the adrenal cortex can be studied.

Distribution and Studies of DDT in the Pregnant Rabbit

After administration of p,p'-DDT to pregnant rabbits, DDT and its metabolites were found in high concentrations in both the fetus and the organs of the maternal rabbit which regulate the fetal environment. p,p'-DDT did not rapidly leave the fetal tissue, as whole body levels at day 26 (17 days after DDT dosing) were higher than those in fetal plasma; fetal whole body levels were also higher than those found in placenta or maternal plasma, and half of those of maternal liver. p,p'-DDT treatment of pregnant rabbits at doses of 10 and 50 mg/kg caused an increased incidence of prematurity, an increase in the number of fetal resorptions and a decrease in fetal weight. These doses of DDT did not increase the incidence of chromosomal aberrations in the maternal or fetal tissue and exposure in vitro to levels as high as 100 µg/ml caused no abnormalities.

Disposition of ^{14}C-Guanazole

The disposition of ^{14}C-guanazole has been examined in the mouse, rat and dog. Guanazole was rapidly excreted in the urine of mice and rats after parenteral administration with 90% of the dose found in mouse urine two to three hours following parenteral administration. Excretion of guanazole by the anesthetized dog was somewhat slower than that by the mouse or rat with 80% appearing in the urine within 6 hours. No evidence was found for the metabolism of guanazole and apparently this antitumor agent is excreted unchanged in these three species. The rapid excretion of guanazole offers an explanation for its greater antitumor activity when given frequently rather than on a once a day schedule.

Studies of ^{14}C-guanazole in man revealed similar findings to the above in that 60% of the radioactivity was found at the end of 24 hours infusion and 91% recovered after 80 hours. Again chromatographic studies revealed that the majority, if not all the radioactivity found, was unchanged guanazole.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the metabolic fate of various anticancer agents, their plasma t 1/2, their route of disposition, and their interaction with other drugs will lead to better therapeutic treatment in man and will aid in the design of agents for experimental animal trials. A rational approach to the design, selection and testing of new anticancer agents is of utmost importance and supplements the screening approach of Drug Research and Development Branch. The possible embryotoxic and/or teratogenic effects of the various anticancer and antiviral drugs is of clinical importance and also may help shed light on nutritional requirements of fetus and on possible mechanisms of contraception. Careful evaluation of the effect of potential and known antitumor compounds in various tumor systems should be made until sufficient data is available to judge their usefulness in screening compounds for ultimate clinical trial.

Proposed Course:

1. Studies of the metabolic fate of new anticancer agents in animals and man will continue. In particular, studies will be intensified on the disposition of alanosine and an acridine derivative, NSC-141,549.

2. Studies on the mechanism of the antitumor action of interferon will continue and the antitumor activity of various interferon inducers and interferon *per se* will be evaluated in various models.

3. A rational approach to the design, selection, and testing of potential anticancer agents will continue.

4. The role of the lymphatic system in drug disposition in general and cancer chemotherapeutic agents in particular will be investigated.

5. Studies of the embryotoxic effects of newer anticancer agents will continue.

6. Use of induced tumors (primary and transplanted) for chemotherapy will be continued.

Publications:

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2. Hart, M. M., Whang-Peng, J., Sieber, S. M., Fabro, S., and Adamson, R. H.: Distribution and effects of DDT in the pregnant rabbit. *Xenobiotica* 2: 567-574, 1972.

3. Hart, M. M., Reagan, R. L., and Adamson, R. H.: The effect of isomers of DDD on the ACTH-induced steroid output, histology and ultrastructure of the dog adrenal cortex. Toxicol. Appl. Pharmacol. 24: 101-113, 1973.

4. Adamson, R. H., and Hahn, M. A.: The disposition of the antitumor agent 3,5-Diamino-1,2,4-triazole (Guanazole) in mice, rats, and dogs. Xenobiotica, 1973, in press.

5. Sieber, S. M., Whang-Peng, J., Johns, D. G., and Adamson, R. H.: The effects of hycanthone on rapidly proliferating cells. Biochem. Pharm., 1973, in press.

Serial No. NCI-3509
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Carcinogenesis, Chemotherapy and Biological Markers in Primates

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Pelayo Correa

Other Investigators: Jacqueline Whang-Peng, T. Phillip Waalkes, Dan Dalgard, Robert McIntire, and Dharam V. Ablashi

Cooperating Units: Laboratory of Pathology, Viral Leukemia and Lymphoma Branch and Human Tumor Cell Biology Branch, NCI; Hazleton Laboratories, Inc. Falls Church, Va., and Bionetics Research Laboratories, Kensington, Md.

Man Years:

Total:	3.0
Professional:	2.0
Others:	1.0

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic in man and to develop model primate tumor systems predictable for therapy for man and for development of biological markers. Specifically the objectives are:

To study the effects of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

To develop model non-human primate tumor systems.

To develop biological markers which will aid in the diagnosis of preneoplastic changes as well as for existing neoplasia.

To evaluate the long-term effects of anticancer drugs which are being used clinically for long-term remissions and for diffuse collagen disorders.

To evaluate known and suspected oncogenic virus in subhuman primates and to utilize these tumors for chemotherapy with antiviral and antitumor agents.

To use normal and tumor-bearing primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To use the various tumors and animals with premalignant changes and with tumors for biochemical and immunological studies.

Methods Employed:

Usual pharmacologic, biochemical, clinical, and anatomical pathologic techniques and cytological and immunologic techniques as well as breeding and nursing care of infant monkeys and special dietary regimens. The breeding record and health status of the breeding colony is similar to that reported for previous years.

Major Findings:

General Information:

The present colony consisting of 600 animals is comprised of five species: Macaca mulatta, Macaca irus, Cercopithecus aethiops, Galago crassicaudatus panganiensis, and Aotus trivirgatus. Of these 450 are experimental; the remaining 150 are adult breeders which supply the newborns for experimental study.

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed in the breeding colony and information concerning the methods utilized in rearing of the newborns. These procedures have not been significantly altered and are, therefore, not included in this report.

The monkeys are housed in an isolated facility which contains only animals committed to this study. Most animals with the exception of the breeding colony are housed in individual cages. As newborns are produced by the breeding colony, they are delivered to the nursery area which is staffed on a 24-hour basis. Compound administration is generally initiated immediately or in the case of the nitrosamines after the monkeys are alpha-fetoprotein (AFP) negative for two weeks as determined by double diffusion techniques.

Various physical hematological and chemical parameters are monitored in each animal to evaluate the general health status and any evidence of tumorigenesis. Surgical biopsies of tumor specimens are obtained when indicated and submitted to the Laboratory of Pathology for histopathologic evaluation. All animals which die or are sacrificed are carefully necropsied and tissues examined microscopically.

No major disease problems have been encountered during this period.

Compounds of Particular Interest:

N-Nitrosodiethylamine (DENA)

This compound has continued to demonstrate potent carcinogenic properties. Administration by the intraperitoneal route continues to produce a very high incidence (100%) of liver tumors which metastasize readily to the lungs and lymphatic tissues. The induction time is inversely affected by the level of administration and the frequency of administration, but generally ranges from 13 to 20 months. The shortest induction time has been eight months.

To date, liver tumors have been induced in over 90 animals utilizing DENA. Sixty-four of these have resulted following intraperitoneal injections generally given every 14 days at a maximum level of 40 mg/kg. The oral administration of DENA at the same level, but given five times weekly resulted in a lower incidence and longer induction time. A total of 30 hepatic cell carcinomas were induced by this method.

Thirty rhesus and cynamolgus newborn monkeys given i.p. DENA after the newborn monkey has been serum alpha-fetoprotein (AFP) negative for two consecutive weeks (generally by age of 1 month) developed tumors by 13 months and evidence of tumor was preceeded in all these animals by the presence of AFP generally 6 months before any evidence of tumor. Six monkeys were sacrificed when AFP positive and upon gross examination of cut sections and microscopic examinations of 100-300 sections no evidence of tumor was found. In all other monkeys in this group with a positive AFP no tumor was found during a laparotomy and biopsy of the liver. Several of these monkeys have subsequently developed tumor even though DENA administration was discontinued following laparotomy. Therefore AFP is a predictive test for monkeys that will eventually have tumor and also indicates a precancerous change which may or may not be susceptible to immunotherapy or prophylactic chemotherapy.

Chemotherapeutic control of tumors has been attempted. L-asparaginase, gallium, methotrexate, camptothecin, and poly I·poly C have been given to several animals. Total regressions were not affected, but evidence of growth suppression by both gross measurements, AFP levels and histopathology has been seen using L-asparaginase, gallium, and methotrexate.

1-Nitrosopiperidine (PIP)

Eight out of ten monkeys receiving PIP orally at a dose of 400 mg/kg five times a week have developed liver tumors after periods of 36 to 89 months on compound. Seven of the eight monkeys were tested for AFP when diagnosed for tumor following biopsy and five had a positive serum AFP as determined by double diffusion.

N-Isopropyl-α-(2-methylhydrazino)-p-toluamide (Procarbazine)

Procarbazine given subcutaneously and orally has induced myelogenous leukemia in two monkeys which received the compound for 17 months and 5 1/2 years respectively. In addition, two monkeys have died with hyperplastic bone marrow, three with ulceration of the esophagus and three with hyperplasia of the urogenital tract.

Attempts to transmit these leukemias by intracerebral inoculation in newborn Rhesus monkeys (M. mulatta) have failed, although this method has been successful for transplanting monkey hepatoma after growth in tissue culture. No viruses have been detected in either of the leukemias by electron microscopic examination of tissue, by animal inoculation or tissue culture. In addition procarbazine induced a malignant hemangiosarcoma in a monkey receiving this compound for seven years.

Aflatoxin B_1

Primary liver carcinoma occurred in two monkeys this past year as a result of giving aflatoxin B_1 orally and i.p. The first tumor resulted in a monkey fed 500 mg over a six year period and the serum was AFP positive. The second tumor occurred in a monkey given less than 100 mg of aflatoxin B_1 for four years generally by i.p. injection. The serum was AFP positive by double diffusion and was 38 μg/ml by radioimmunoassay. This tumor was resected and weighed 12.9 grams.

Two weeks following resection the AFP became negative by double diffusion and is currently 100 ng/ml as determined by radioimmunoassay. Cells from the tumor are presently growing in vitro and producing AFP.

Cycasin

One monkey fed cycasin and its aglycone (MAM acetate) for over seven years developed a well differentiated liver cell carcinoma. The tumor is presently growing in tissue culture and producing AFP.

Other Carcinogens

Monkeys receiving other carcinogens have thus far not developed tumors although preneoplastic lesions have been seen in monkeys receiving

methylnitrosourea and the polycyclic hydrocarbons. Other lesions seen with monkeys receiving fluorenylacetamides, azodyes, ethyl carbamate and the artificial sweetners remain the same as detailed in annual reports in prior years.

Herpesvirus Saimiri (HVS)

Thirty owl monkeys inoculated with HVS propagated in owl and African green monkey kidney cell cultures developed malignant lymphoma with lymphocytic leukemia. The disease had a course of 55 to 80 days and was characterized by generalized lymphadenopathy and lymphocytic leukemia with circulating actively motile lymphocytes. The lesions were characterized by the obliteration of normal lymphoid tissue architecture by neoplastic lymphocytes and by neoplastic lymphocytic invasion of almost all organs. No virus was detected in the tissue by electron microscopy, however, upon co-cultivation HVS was recovered.

Chemotherapy of owl monkeys with malignant lymphoma and lymphocytic leukemia has been continued and results suggest that cytoxan, cytosine arabinoside, vincristine and steroids and human interferon are effective in the treatment of owl monkeys with this disease. In addition several agents were examined for their effects on the multiplication of HVS in vitro. The multiplication of HVS in vitro was inhibited by cytosine arabinoside adenine arabinoside, and tilorone, but not by rifamycin SV.

Alpha-Feto Protein Studies

With the use of the relatively insensitive double diffusion technique which allows detection of AFP in the range of 500 ng to 1 µg/ml we are able to detect AFP well in advance of any histological evidence of tumor - generally tumor can be predicted in an animal 6-8 months prior to histological evidence. In addition in a co-operative study with Dr. McIntire the serum AFP has been quantitated using a sensitive double-antibody radioimmunoassay. This technique allows us to detect nanogram amounts of AFP - the control value for monkeys being in the range of 10 ng/ml.

The serum levels in biopsy-proven disease were scattered over a wide range of 100,000 fold variation, which is analogous to the range seen in human hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine induced neoplasms had significantly higher levels of AFP than those induced with 1-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations of AFP levels demonstrated a tendency for each monkey with liver tumor to plateau after reaching a level characteristic for that tumor.

Catabolic rate of AFP was measured using both purified and non-purified material. The T 1/2 with native protein was between 3 and 4 days. In vitro synthetic studies on biopsy-derived monkey liver tumors indicate that these cells synthesize and secrete AFP at a steady rate for 4-6 days. This provides a model system for calculating total body synthesis of AFP and the average cellular synthetic rate and thereby estimate the total number of liver tumor cells in the body.

Diagnostic Studies

Diagnostic studies for primary hepatocellular carcinoma which may be applicable to human primary and secondary liver cancer have continued using ^{67}gallium administered i.v. and experiment X-ray contrast materials administered via the hepatic artery. Studies to date indicate that these materials may well be useful in the diagnosis of primary and secondary liver tumors and for careful evaluation of effects of anticancer drugs on liver tumors.

Significance to Bio-medical Research and the Program of the Institute:

Tumors induced in non-human primates are likely to be more useful than the rodent tumor system now used to predict the usefulness of anticancer agents for man. In particular, the owl monkey may serve as a useful model for lymphoma and leukemia and for combination therapy with antiviral agents. The rhesus induced hepatoma system appears to be an excellent model for human hepatoma both with regard to response to therapy and AFP synthesis. Potential diagnostic tests for precancerous and malignant tissue may also be discovered by use of non-human primate tumors; few such tests now are useful for man. The usefulness of immunotherapy in the treatment of precancerous as well as frank neoplasia can be ascertained with the DENA induced precancerous and liver tumor. In addition the susceptibility of non-human primates to known rodent carcinogens and to possible carcinogens in man is of theoretical, practical, and economic importance. In addition, studies of the carcinogenic potential of antitumor agents will indicate whether these compounds should or should not be used clinically in patients without neoplastic disease for organ transplantation and in treating arthritis and other diffuse collagen disorders. The susceptibility of the newborn monkey vs. the adult monkey to carcinogens and other foreign compounds is information that is needed for the clinician and for judging the safety of various food additives, supplements, and other compounds to which man is exposed.

Proposed Course:

1. Treatment with present carcinogens will be continued in the monkey, and it is hoped that an observation period of 10-15 years will give some basis for predicting which compounds are likely to be carcinogenic in man.

2. Treatment of monkeys with liver tumors will be continued with various chemotherapeutic agents given i.v. and by intraarterial infusion.

3. Studies will continue on the correlation of the production of AFP in monkeys with the time of appearance of tumors and with histopathologic changes in the liver and in the tumors.

4. More owl monkeys will be inoculated with HVS and when disease is apparent treatment with other antileukemic and antiviral agents will be undertaken. In addition, prophylactic chemotherapy will be initiated.

5. A continued effort will be made to assess the carcinogenic potential and long term toxicity of procarbazine and other antitumor agents being used for treatment of non-neoplastic disease.

6. Various monkeys will be utilized for comparative disposition studies and toxicological evaluation of anticancer, antiviral, and antimicrobial agents.

7. Studies of the metabolism of the various classes of carcinogens in various species will be continued. Such studies, it is hoped, will give more insight into susceptibility or refractoriness of primates compared to rodents.

8. Attempts will be made to develop primate tumor models of colon cancer and stomach cancer. Potential biological markers for various types of tumors will be investigated.

Publications:

1. O'Gara, R. W., and Adamson, R. H.: Spontaneous and induced neoplasms in nonhuman primates. In Fiennes, R-N. T-W. (ed): Pathology of Simian Primates. Basel, New York, S. Karger, 1972, pp. 190-238.

2. Adamson, R. H., Smith, C. F., and Dalgard, D. W.: Induction of neoplasms in nonhuman primates by chemical carcinogens - correlation of serum alpha-fetoprotein and appearance of liver tumors. In Anderson, N. G., Coggin, J. H., Jr., Cole, E. and Holleman, J. W. (ed): Embryonic and Fetal Antigens in Cancer Vol. 2. Feb. 14-16, 1972. Oak Ridge National Laboratory, Tenn., 1973, pp. 331-337.

3. Adamson, R. H., Correa, P., and Dalgard, D. W.: Occurrence of a primary liver carcinoma in a rhesus monkey fed aflatoxin B_1. J. Natl. Cancer Inst. 50: 549-553, 1973.

4. Vogel, C. L., Adamson, R. H., DeVita, V. T., Johns, D. G., and Kyalwazi, S. K.: Preliminary clinical trials of dichloromethotrexate (NSC-29630) in hepatocellular carcinoma. Cancer Chemother. Rep. 56: 249-258, 1972.

5. Adamson, R. H., Ablashi, D. V., Armstrong, G. R., and Ellmore, N. W.: Effect of cytosine arabinoside, adenine arabinoside, tilorone, and rifamycin SV on multiplication of Herpesvirus saimiri in vitro. Antimicrob. Agents Chemother. 1: 82-83, 1972.

Serial No. NCI-6108
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanisms of Action and Mechanisms of Resistance to Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson

Other Investigators: T. Phillip Waalkes, Susan S. Fabro and Richard Cysyk

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design new anticancer agents utilizing knowledge obtained so that a higher cell kill can be obtained and the problem of resistance can be circumvented.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis.

Major Findings: Serial No. NCI-6108

This year we have been concerned with the mechanism of action of interferon per se, hycanthone and analogs and various intercalating agents. In addition, studies have been initiated on the purification of ribonucleotide reductase inhibitors and the design of agents to inhibit this enzyme. Effects of various drugs on t-RNA methylase were also determined.

Effects of Antitumor Agents on t-RNA Methylase

The effect of various antitumor drugs on t-RNA methylase activities of leukemia L1210 cells grown in tissue culture were determined and results were correlated with effects on cell proliferation and growth. Methotrexate, vincristine, and 1,3-bis-chlorethyl-1-nitrosourea (BCNU) depressed cell proliferation, but only methotrexate caused an inhibition of total cellular t-RNA methylase activities. However, the inhibition was not a direct effect since addition of methotrexate to a cell-free system produced no changes in t-RNA methylase activity. The reduction of methylase activity by methotrexate was preceded by an inhibition of protein synthesis, suggesting that the reduced enzyme levels are secondary to a decrease in the biosynthesis of t-RNA methylases. Therefore, the effect of drugs on the level of t-RNA methylase activity does not predict for their effectiveness as antitumor agents.

Interferon

Mouse interferon was found to have antitumor effects against the transplantable tumor A-RCS but not against leukemia L1210 or P388. Furthermore, human interferon was found to have antitumor effects against HVS induced lymphoma-leukemia in owl monkeys. The mechanism of this antitumor effect is not a direct cytotoxic effect since interferon per se is not active in tissue culture. Effects of interferon on macromolecular synthesis are in progress.

Hycanthone

The effect of hycanthone on DNA, RNA and protein synthesis was studied using PHA stimulated human lymphocytes. Protein synthesis was not inhibited at any time interval after addition of PHA whereas both DNA and RNA synthesis were inhibited at various time intervals with maximal inhibition occurring at 30 hours.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the mechanism of action of anticancer agents will lead to the design of newer drugs, hopefully, with greater cell kill and less toxicity. Knowledge of the mechanism of resistance to various anticancer agents will lead to measures that minimize development of resistance or will overcome this resistance. In addition, there is the possibility that resistant cells can be converted into sensitive cells.

Proposed Course:

The mechanism of antitumor activity of several newer anticancer agents, including coralyne, maytansine, gallium, and other metals will be investigated. Further studies with regard to mechanism of resistance to anticancer agents will also be undertaken.

Publications:

None

Serial No. NCI-6116
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Nitroreductase Activity of Hepatic Aldehyde Oxidase

Previous Serial Number: Same

Principal Investigator: David G. Johns

Other Investigators: Mary K. Wolpert

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives:

A number of nitro-substituted N-heterocyclic compounds are active carcinogens; their carcinogenic activity is thought to be due however to the corresponding hydroxyamino metabolite, rather than to the parent nitro compound. Most mammalian nitroreductase activity is found in the liver, and occurs in both microsomal and soluble supernatant fractions from liver homogenates. The soluble supernatant nitroreductases (prominent among which are aldehyde oxidase, xanthine oxidase, and DT-diaphorase) are able to catalyze reduction to the biologically active hydroxyamino stage, while the microsomal nitroreductases appear to be able to carry the reduction further, to the corresponding amino compounds. The objective of this project is to characterize the previously little studied nitroreductase activity of mammalian hepatic aldehyde oxidase, and to assess its quantitative significance in the biological activation of this group of carcinogens.

Methods Employed:

Hepatic aldehyde oxidase is obtained from a number of mammalian species (rabbit, guinea pig, mouse, and rat) and purified by ammonium sulfate fractionation followed by gel filtration, column chromatography, and other standard enzyme purification methods. Nitroreductase activity is assayed spectrophotometrically under anaerobic conditions, using either nitrofurazone

or the carcinogen 4-nitroquinoline-N-oxide as electron acceptor. The products of enzymic reduction are identified by ultraviolet and infra-red absorption spectroscopy, by thin-layer and paper chromatography, and by carbon-nitrogen-hydrogen combustion analysis. Reference compounds are prepared by chemical reduction of the parent nitro compound.

Major Findings:

Nitrofurazone, 2-bromo-5-nitrothiazole, and the carcinogen 4-nitroquinoline-X-oxide were readily reduced by aldehyde oxidase under anaerobic conditions to the corresponding hydroxylamino compounds; a variety of other nitro compounds, including p-nitrobenzoate, did not show detectable acceptor activity. Effective electron donors for the reduction included aldehyde substrates such as butyraldehyde and benzaldehyde, and N-heterocyclic substrates such as 2-hydroxypyrimidine, 3'5'-dichloromethotrexate, and N-methylnicotinamide. NADH and NADPH failed to show detectable electron donor activity. The nitroreductase activity of aldehyde oxidase was significantly inhibited (>50%) by menadione (15 μM) and by Triton X-100 (7×10^{-4}%); in contrast to that of xanthine oxidase, however, it was not inhibited by 4-hydroxypyrazolo(3,4-d)pyrimidine (allopurinol) (5 μM). The ratios of aldehyde oxidase:xanthine oxidase nitroreductase activity in the 105,000 x g supernatant fraction of liver homogenates from several mammalian species, utilizing nitrofurazone as electron acceptor, were as follows: rabbit, 4.8:1; guinea pig, 1.4:1; and rat, 0.9:1.

Significance to Bio-medical Research and the Program of the Institute:

Nitro compounds are widely distributed in the environment, and are readily reduced in vivo to hydroxyamino, nitroso, and amino derivatives. The reduced forms of certain nitro compounds are known to possess activity as carcinogens. Study of the nitroreductase activity of aldehyde oxidase and the assessment of its quantitative significance in nitro group reduction in vivo can thus lead to information of potential relevance to the etiology of neoplastic disease.

Proposed Course:

While our present studies show that hepatic aldehyde oxidase possesses significant nitroreductase activity in vitro, the quantitative role of this reaction in vivo has not yet been determined. Attempts will be made to study the reaction under conditions more nearly approaching those obtaining in vivo, and the effect of specific aldehyde oxidase inhibitors on nitro group reduction and on the carcinogenic activity on nitroquinoline-N-oxide in the whole animal will be carried out.

Publications:

Wolpert, M. K. and Johns, D. G.: Nitroreductase activity of mammalian hepatic aldehyde oxidase. Fed. Proc. 31: 606, 1972.

Wolpert, M. K., Althaus, J. R., and Johns, D. G.: Nitroreductase activity of mammalian liver aldehyde oxidase. J. Pharmacol. Exp. Ther. 184: (May) 1973.

Serial No. NCI-6120
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Kinetic Profile on Drug Action In Vivo

Previous Serial Number: Same

Principal Investigators: Bruce A. Chabner and Robert C. Young

Other Investigators: Charles B. Myers and Steven Rosenoff

Cooperating Units: Solid Tumor Service, Medicine Branch, NCI

Man Years:

Total: 2

Project Description:

Objectives:

In using any pharmacologic agent, certain critical information is needed in order to design an optimal schedule of drug administration. One must know:

1) The nature and kinetics of distribution and disposition of the agent.
2) Its mechanism of action.
3) The levels of parent drug or actor metabolite required for the desired effect on target tissue.

While extensive information is now accumulating with regard to pharmacokinetics and mechanism of action of the common antitumor drugs, little is known about the drug concentrations which produce toxicity and antitumor effects in vivo. This important consideration is the object of our research efforts. We have chosen methotrexate as a model agent for study because it is easily measured and active as the parent compound. Secondly, its mechanism of action, the inhibition of dihydrofolate reductase, with consequent block of the conversion of dUMP to $dTMP_3$ allows one to follow its action in vivo by determining the incorporation of ^{3}H-UdR into DNA.

We have also extended our studies to 5-fluorouracil and are in a position now to determine similar correlations between plasma and intracellular levels of F-dUMP and inhibition of thymidylate synthesis by 5-FU.

Methotrexate Studies

Methods Employed:

Methotrexate (MTX) levels were determined by the standard dihydrofolate-reductase inhibition assay, which allows quantitation of MTX levels of 10^{-9}M or greater. BDF_1 and CDF_1 mice, some bearing L1210 ascites tumor, were injected with 5, 50, or 350 μg/kg MTX and were sacrificed at frequent intervals. One hour prior to sacrifice ^{3}H-UdR was injected i.p. in tracer amounts and the incorporation of label into DNA of bone marrow, intestinal epithelium, and ascites tumor was determined by phenol extraction and scintillation counting.

Major Findings:

1. The extended pharmacokinetics of methotrexate in mice have been defined using the highly specific dihydrofolate reductase assay. The persistence of MTX in plasma for several days at concentrations of 10^{-8}M or greater has been demonstrated following doses of 350 mg/kg (LD_{30}) or greater. A third phase ½ life of ∼ 12 hours has been defined in mice. This phase is not predicted by current pharmacokinetic models of MTX disposition.

2. At all dose levels, >50% inhibition of ^{3}H-UdR incorporation into DNA was observed in all tissues when plasma levels were 10^{-8}M or greater, while rapid recovery of this biochemical function took place when plasma [MTX] fell below this figure. Recovery of bone marrow took place at $1x10^{-8}$M MTX while recovery of intestinal epithelium occurred at $5x10^{-9}$M MTX or less.

3 The recovery of bone marrow clearly antedated recovery of intestinal epithelium at the two higher dose levels of the drug, thus explaining the dose limiting nature of intestinal toxicity of MTX in mice.

In summary, these studies have shown that each dose level of MTX is associated with a characteristic kine ic profile of biochemical effect which is intimately related to the pharmacokinetics of the drug. In particular, inhibition of ^{3}H-UdR incorporation into DNA was maintained in host tissues so long as plasma levels of MTX remained above 10^{-8}M.

Proposed Course:

1. Extend studies to man. We have performed serial analyses of ^{3}H-UdR incorporation into bone marrow and ascites tumor in 2 patients with ovarian carcinoma and in general have found a similar threshold for inhibition of bone marrow DNA synthesis in man. Tumor sensitivity has varied widely, however, and further studies are planned to obtain a clearer idea of this aspect of the work. We have noted elevated levels of MTX in ascites fluid as compared to plasma and plan to study this with regards to the presence of a folate binding protein in ascites.

2. Define pharmacokinetics of the third phase of MTX plasma disappearance in man. We suspect the persistence of the drug in man is related to renal reabsorption of MTX and plan to perform careful renal clearance studies in patients receiving MTX.

5-Fluorouracil Studies

Methods Employed:

Kinetic studies similar to those performed with MTX were undertaken with 5-FU in mice with a sensitive tumor, P1534, and a resistant tumor, Ehrlich ascites.

Major Findings:

1. A dose dependent inhibition of ^{3}H-UdR incorporation into DNA in both normal and tumorous tissues. The duration of inhibition was much greater in the sensitive as opposed to resistant tumor.

2. Increased sensitivity of normal tissues to second doses of 5-FU during the recovery phase.

3. Exploitable kinetic differences in the recovery of normal and tumorous tissues such that repeat doses of 5-FU can be given with maximum effectiveness when tumor is in the recovery phase and normal tissues are fully recovered and returned to basal levels of ^{3}H-UdR incorporation.

These results are discussed more fully in the Medicine Branch Annual Report by Dr. Robert C. Young (Serial No. NCI-3420).

In addition, work has proceeded on the development of a sensitive enzymatic assay for the active 5-FU metabolite, F-dUMP. Dr. Charles Myers has perfected a very sensitive assay using L. casei thymidylate synthetase and has shown the ability of the enzyme to detect F-dUMP in tissue samples at levels approaching 2×10^{-9}M. This assay also allows measurement of dUMP pool size in tissue, a useful parameter in studies of ^{3}H-UdR incorporation.

Proposed Course:

1. Determine correlation of F-dUMP levels and inhibition of ^{3}H-UdR incorporation following 5-FU in tumor and normal tissues of tumor-bearing mice.

2. Extend these observations to man to determine whether such correlation exists for inhibition of DNA synthesis in bone marrow and in tumor cells.

Publications:

Chabner, B. A. and Young, R. C.: Threshold methotrexate concentration for inhibition of DNA synthesis in normal and tumorous tissues. J. Clin. Invest., 1973, in press.

Presentation:

See Clinical Pharmacology and Cellular Kinetics sections of the Medicine Branch Annual Report (Serial No. NCI-3420).

Serial No. NCI-6121
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Purification and Properties of Carboxypeptidase G_1

Principal Investigator: Bruce A. Chabner

Other Investigators: Joseph R. Bertino, David Makulu, David G. Johns, and Robert C. Young

Cooperating Units: Department of Pharmacology, Yale University School of Medicine; New England Enzyme Center of Tufts University; Solid Tumor Service, Medicine Branch

Man Years:

Total: 1

Project Description:

Objectives:

1. To simplify and improve the purification of carboxypeptidase G_1 (CPD G_1) in order to allow large scale production of this enzyme.

2. To determine the antitumor activity of CPD G_1 in mice.

3. To determine the toxicity of CPD G_1 in mice, dogs, and monkeys.

4. To begin clinical trials of this agent if warranted by animal data.

5. To investigate prevention of methotrexate toxicity by CPD G_1.

Results:

CPD G_1, a folate-cleaving enzyme from Pseudomonas stutzeri, has been purified to homogeneity by a series of ion exchange, gel filtration, and electrofocusing steps.

Mr. Henry Blair, New England Enzyme Center, Tufts University, and Dr. Chabner from this department, have worked outa purification procedure for CPD G_1 allowing preparation of homogeneous enzyme in large quantities. The method involves attachment of crude enzyme to CM-sephadex at pH 6.0 and specific elution with Na-glutamate. An improved fermentation technique has also been worked out using 10-fold increases in substrate concentration and yielding

a 10-fold greater quantity of enzyme. Under contract with this section, the New England Enzyme Center has provided us with 200,000 units of highly purified CPD G_1, which will be tested shortly for clinical activity at Yale University by Dr. Joseph Bertino. Further clinical studies of this agent will await support for additional enzyme fermentation and purification.

Other research studies on CPD G_1 this year have included the following:

1. Demonstration in vivo that therapeutic doses of CPD G_1 have no effect on DNA synthesis in bone marrow and GI epithelium, while dramatically inhibiting both UdR and TdR incorporation in L1210 tumor cells. This effect on TdR incorporation into DNA differs strikingly from the effect of methotrexate on murine and human neoplasms, in that only UdR incorporation is suppressed initially by MTX.

2. Rabbit anti-CPD G_1 antibodies have been developed in response to CPD G_1 administration subcutaneously in Freund's adjuvant. These antibodies are capable of inactivating CPD G_1 in vitro and depress CPD G_1 plasma levels when administered passively to mice, but this depression appears reversible with time in that a secondary rise in plasma CPD G_1 levels is seen 6 hours after antibody administration. No antibody has been detected in dogs or mice given CPD G_1 on acute or chronic administration schedules.

3. CPD G_1 activity in vivo has been shown by its ability to cleave circulating methotrexate and to "rescue" animals from lethal methotrexate toxicity if given 24 hours after the antifolate. These experiments were published in Nature this year and represent the first demonstration of drug metabolism in vivo by passively administered enzyme. A more complete description of these studies is given in the 1971-1972 Annual Report.

Publications:

1. Chabner, B. A. and Bertino, J. R.: Activation and inhibition of carboxypeptidase G_1 by divalent cations. Biochim. Biophys. Acta 267: 234-240, 1972.

2. Chabner, B. A., Chello, P. and Bertino, J. R.: Antitumor activity of a folate-cleaving enzyme, carboxypeptidase G_1. Cancer Res. 32: 2114-2119, 1972.

3. Chabner, B. A., Johns, D. G., and Bertino, J. R.: Enzyme cleavage of methotrexate: A novel method for reversal of drug toxicity. Nature 239: 395-398, 1972.

Serial No. NCI-6124 (New)
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical and Pharmacological Studies Designed to Improve Clinical Cancer Chemotherapy: 1) Mechanism of Action of Select Tumor Agents; 2) Biochemical Markers of Malignancy; 3) Enzymatic Reactions which may be Exploited for the Development of Better Chemotherapeutic Agents

Previous Serial Number: NCI-6118

Principal Investigator: T. Phillip Waalkes

Other Investigators: Richard H. Adamson and R. Graham Smith

Cooperating Units: Laboratory of Tumor Cell Biology, NCI

Man Years:

Total:	3
Professional:	1 1/4
Other:	1 3/4

Project Description:

Objectives:

1. To obtain information regarding the biochemical and pharmacologic mechanisms whereby chemotherapeutic agents, known to be at least partially effective against various malignancies in man, appear to act. It is considered that information of this type will be helpful not only in the selection of possible analogs, the synthesis of specific compounds, and a rational approach to combination therapy, but also in the understanding of the basic biochemical processes of malignancy and malignant cell growth. The project completed during the previous year involved the study of tRNA methylation in specific neoplastic systems and the influence of selected chemotherapeutic agents. Various reports have confirmed the finding that hypermethylation of tRNA is a characteristic of the tumor cell. Although the function served by the addition of methyl groups to the nucleic acid bases of tRNA is not clear, the important role of tRNA in protein synthesis lends further emphasis to the possibilities.

2. The Development of a Biologic Markers Program. Because of the need for sensitive methods for determining not only the presence of malignant cells but also their number in any patient with cancer, a Biologic Markers Program has been initiated. The Program objective is to develop specific and sensitive techniques to detect and to measure biochemical or biological materials which are quantitative indicators of the cancer cell. Such markers are important in the evaluation of response to therapy, in determining the course of disease, and in estimating the amount of residual tumor.

3. Specific enzyme reactions involving RNA and DNA have been studied from a variety of approaches. In particular, RNA dependent DNA polymerase and DNA dependent DNA polymerase in several different tissue and types of cells have been evaluated as to their relationship to tumor cell growth and proliferation. Primary emphasis was placed on the separation, isolation, characterization of the DNA Polymerases of Walker-256 carcinoma, a tumor frequently used to study antitumor agents. The study of specific antitumor drugs as enzyme inhibitors was carried out.

Methods Employed:

1. The L1210 system both in vitro (tissue culture) and in vivo was used to evaluate the effects of specific chemotherapeutic agents on the methylation of tRNA. The agents studied included methotrexate, vincristine, BCNU, poly IC, 6-MP and MIH.

2. Three types of analytical techniques are being used: (1) High-pressure liquid ion exchange chromatography in conjunction with such analytical tools as GC (gas chromatography), GC-MS (gas chromatography-mass spectrometry), and high and low resolution MS; (2) Gas-liquid chromatography (GLC); and (3) Radioimmunoassay. Four contracts have been established to carry out the various types of assays, with the Oak Ridge National Laboratories for high-pressure liquid chromatography, the University of Missouri for GLC, and Brandeis University for radioimmunoassays. These contracts complement the in-house research efforts.

3. Because of in vivo tumor inhibition by certain chemotherapeutic agents which are also anti-viral compounds, Ca-256 cells were studied for DNA polymerase activity and to detect the possible existence of a reverse transcriptase. Standard techniques with radioactive labeled compounds were used to study the in vitro kinetics of the enzymatic reactions. Purification was carried out with high speed centrifugation, DEAE chromatography, phosphocellulose chromatography, and glycerol gradient centrifugation.

Major Findings:

1. In vitro, methotrexate had a definite inhibitory effect on methylation at 24 to 48 hours. None of the other compounds showed a similar effect. Further studies indicated that methotrexate also caused a profound inhibition on protein synthesis in the same system under similar conditions. This effect may well be the explanation for the changes found in the decreased methylation of tRNA. The results of this project have now been published.

2. Studies are in progress to develop methods and to evaluate whether or not specific materials are elevated in the blood or urine of cancer patients. Initially, patients with advanced disease have been entered into these studies. Preliminary results indicated that specific tRNA degradation products are increased in the urine of some patients with cancer. Further studies are necessary to determine the extent of these elevations and the types of tumors associated with increased excretion. Polyamines appear to be slightly elevated in certain cancer patients urines. Further studies regarding methodology are necessary before final conclusions can be reached. To date studies by radioimmunoassays for methylated nucleic acid bases in serum have been disappointing. Sufficient sensitivity as yet has not been reached in order to detect these compounds by this technique.

3. Several DNA polymerases were isolated including a reverse transcriptase, as judged by current criteria. These polymerases were all purified and characterized. Antitumor compounds were tested as possible inhibitors using in vitro assays. The results showed individual differences in the inhibition of the specific polymerases, but complete correlation with in vivo results was not evident.

Significance to Bio-medical Research and the Program of the Institute:

1. The results showed no correlation between inhibition of in vivo tumor growth and of tRNA methylase activity by the various antitumor agents using this in vitro system, implicating that the mechanism of action of these compounds was not mediated through inhibition of tRNA methylation. As a method for screening potential antitumor agents, tRNA methylation does not appear to be a promising enzymatic reaction.

2. It is of considerable importance to cancer clinical research and to cancer patients to develop more sensitive means for determining the presence of malignant cells and to develop methods for estimating the number of these cells present and/or remaining after therapy. The Biologic Markers Program objective is to develop methods of assay to evaluate promising materials as potential markers, and to determine their usefulness during actual clinical studies.

3. The role of reverse transcriptase has not been clarified. Its presence in a common rat tumor used frequently for assessing antitumor agents is of significance. The fact that several of the compounds which inhibit the growth of Walker-256 also inhibit specific viruses causes speculation as to the possibility that the growth of this tumor may be dependent on viral reverse transcriptase or that the tumor was originally initiated by a virus.

Proposed Course:

1. No further studies of the inhibition of tRNA methylase activity in tumor cells by antitumor agents are being considered.

2. Future efforts will largely be focused on the Biologic Markers Program. Plans are in process to increase the scope of the current initial program. A greater number of potential markers will be studied. Evaluations will be carried out for cancer patients before, during, and after therapy. In addition, efforts will be made to develop practical methods for determining the number of tumor cells present in patients by means of appropriate techniques.

3. No plans at present are contemplated for extending the work with polymerases of the Walker-256 tumor.

Honors and Awards: None

Publications:

1. Waalkes, T. P., Adamson, R. H., Riddick, D., and Gallo, R. C.: Evaluation of antitumor agents on tRNA methylase activity of leukemia L1210. Arch. Int. Pharmacodynamie et Therap., 196: 345-351, 1972.

2. Waalkes, T. P., Dinsmore, S., and Mrochek, J.: The presence of the nucleosides, N^2,N^2-dimethylguanosine, 1-methylinosine, and pseudouridine in the urine of cancer patients. J. Nat. Cancer Inst., in press.

3. Gehrke, C. W., Kuo, K. C., Zumwalt, R. W., and Waalkes, T. P.: The determination of polyamines in urine by gas-liquid chromatography. In Russell, D. H. (ed.): Polyamines in Normal and Neoplastic Growth. New York, New York, Raven Press, 1973, in press.

Serial No. NCI-6125 (New)
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pharmacology of 2,6-Dimethyl-4-benzyl-4-demethylrifampicin (NSC-143414) and other Inhibitors of Viral RNA-directed DNA Polymerase

Previous Serial Number: None

Principal Investigator: David G. Johns

Other Investigators: Mary K. Wolpert

Cooperating Units: Department of Developmental Therapeutics, M.D. Anderson Hospital and Tumor Institute, Houston, Texas

Man Years:

Total:	1.2
Professional:	0.8
Other:	0.4

Project Description:

Objectives:

2,6-Dimethyl-4-benzyl-4-demethylrifampicin (DMB; NSC-143414) inhibits viral RNA-directed DNA polymerase, delays the onset of carcinogen-induced mammary tumors in rats, and inhibits the transformation of UC1-B cells by murine leukemia virus. Because of the potential usefulness of DMB in cancer chemotherapy in man, animal pharmacology studies have been initiated. Absorption, distribution, plasma clearance, metabolism and excretion of the drug will be assessed in several animal species as an aid to the design of optimal therapeutic dosage regimens. Similar studies will be carried out with other viral RNA-directed DNA polymerase inhibitors as they become available.

Methods Employed:

DMB in biological fluids is assayed by a spectrofluorometric method or by liquid scintillation counting of drug labeled with ^{14}C in the 38 (formyl)-position of the side-chain. DMB is administered orally and intravenously to mice, rats, dogs and monkeys, and plasma, urine, cerebrospinal fluid, biliary and fecal levels of the drug are determined. Metabolites are separated from the parent drug by thin-layer chromatography, and the ratios of unaltered to

metabolized drug in biological fluids ascertained. Tissue levels of the drug are determined by the combustion method followed by scintillation counting of $^{14}CO_2$, or by dissolving the tissue with the aid of solubilizing agents prior to addition to liquid scintillation counting medium.

From the data obtained, the dose-levels and frequency of administration required to give therapeutic plasma and tissue levels of the drug (5 - 50 μg/ml) are ascertained. Pharmacokinetic models are developed to permit extrapolation of the data obtained to man.

Major Findings:

DMB was administered to dogs at 1 or 5 mg/kg either orally or intravenously. Fluorometric assays showed that plasma DMB concentration fell sharply within 1 hour, with a t-1/2 of 45 min., then stayed nearly constant from 2 to 5 hours. Significant plasma levels were observed after oral administration. In rats, 24 hours after i.v. injection of 10 mg/kg of DMB-formyl-^{14}C, high tissue/plasma ratios were found in liver (6.0), pancreas (3.7) and adrenals (7.1). Tissues with low ratios included fat (0.6), muscle (0.7) and brain (0.1). In 3 hours, 2% of the drug was excreted in bile; a peak bile/plasma ratio of 2.9 was achieved at 1 hour. Fecal excretion amounted to 27% of the dose over 5 days. Thin-layer chromatography of rat liver and fecal extracts indicated that most of the ^{14}C was present as the parent (hydroquinone) and quinone forms of the drug. Less than 2% of the i.v. dose was found in urine 5 hours after 1 mg/kg in dogs and 24 hours after 10 mg/kg in rats. In the mouse, no $^{14}CO_2$ could be detected in expired air within 6 hours after 10 mg/kg of the labeled drug.

Significance to Bio-medical Research and the Program of the Institute:

Of the numerous compounds which have been examined for activity as inhibitors of viral RNA-directed DNA polymerase ("reverse transcriptase"), the rifampicin series appear to offer greatest promise in terms of specificity and potency. The therapeutic trial of these and other inhibitors of the viral transformation of cells for their activity in remission maintenance in human neoplastic disease is of great current interest to the program of the Institute. The role of this research project is to provide vital whole animal pharmacology data which will be of value in designing rational dose-schedules for the use of these agents in man.

Proposed Course:

Whole-animal pharmacology studies will be continued, and sufficient further data will be collected to permit soundly based extrapolation of these pharmacological data to man. The significance of plasma protein binding in limiting the therapeutic efficacy of DMB and other members of the rifampicin group *in vivo* will also be ascertained.

If clinical pharmacological and Phase I studies with these agents are initiated, methodology developed for use in whole animal studies will be utilized for the determination of plasma and urine levels in man.

Publications:

Wolpert, M. K., Lu, K., Althaus, J. R., Johns, D. G., and Loo, T. L.: Absorption, distribution and excretion of 2,6-dimethyl-4-benzyl-4-demethyl-rifampicin in rats, mice and dogs. Fed. Proc. 32: 777, 1973.

Serial No. NCI-6126 (New)
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Purification and Properties of Cytidine Deaminase

Principal Investigators: Bruce A. Chabner and David G. Johns

Other Investigators: None

Cooperating Units: Hematology and Supportive Care Branch, Plasmaphoresis Laboratory; Medicine Branch

Man Years:

Total: 2

Project Description:

Objectives:

1. To purify and characterize the enzyme cytidine deaminase found in normal and malignant leukocytes.

2. To elucidate the mechanism of enzyme regulation and its induction by cytosine arabinoside *in vivo*.

3. To explore means of utilizing the induction of this enzyme for the production of lethal pyrimidine antagonists *in vivo*.

The enzyme cytidine deaminase is known to inactivate cytosine arabinoside. Recent studies have shown that increased levels of this enzyme may be induced by ara-C in patients with acute myelocytic leukemia, and that increased deaminase is likely responsible for the development of ara-C resistance in these patients. Little is known about this enzyme in AML cells at present; thus we have undertaken detailed studies of its biochemical properties in hopes of characterizing its substrate requirements and regulation in leukemic cells.

Methods:

1. Assay: Cytidine deaminase activity (CD'se) was determined by separation of product and substrate on Dowex 50 H^+ resin. An alternate method was developed for studies of 5-azacytidine and halogenated pyrimidines using a coupled assay with glutamic dehydrogenase to measure NH_4^+ production.

Purification procedures were standard biochemical usage of $(NH_4)_2SO_4$ fractionation, Sephadex gel filtration, electrofocusing, and ion exchange chromatography.

Results:

1. Thirty-fold purification has been accomplished. A molecular weight of 50,000 has been established for CD'se from both normal and leukemic granulocytes.

2. Enzyme Kinetics: Km for cytidine is $1x10^{-5}M$; for ara-C, $1x10^{-4}M$; and for 5-azacytidine, $4.3x10^{-4}M$. Other substrates include 5-fluorocytidine, 5-Iodo-2-deoxycytidine, and deoxycytidine. Cyclocytidine is neither a substrate or inhibitor.

3. Tetrahydrouridine is an effective competitive inhibitor with a Ki of $5x10^{-7}M$.

4. Enzyme levels are greatest in normal mature granulocytes and are lowest in AML cells, suggesting a relationship between cell maturation and enzyme level.

5. The new antitumor agent, 5-azacytidine, is also a substrate for this enzyme, suggesting the possibility of clinical cross resistance to these two agents on the basis of ↑ deaminase activity.

Proposed Studies:

1. Further studies on purification.

2. Studies of mechanism of regulation and induction in tissue culture.

3. Studies on relationship of granulocyte maturation and CD'se in normal human granulocyte precursors.

4. Possible use of fluorinated cytidine derivatives to produce 5-FUdR or 5-FUR in treating tumors with high levels of CD'se.

Presentation:

Isolation and characterization of cytidine deaminase from human leukemic leukocytes. Amer. Assoc. Cancer Res., Atlantic City, N.J., April 12, 1973.

SUMMARY REPORT

LABORATORY OF MOLECULAR PHARMACOLOGY

DIVISION OF CANCER TREATMENT

July 1, 1972 -- June 30, 1973

Studies of DNA damage, replication, and repair.

Many chemotherapeutic agents act either directly or indirectly by causing damage to cell DNA, thereby bringing about either cell death or inability of the cell to multiply. Methods for detecting early stage of DNA damage therefore are of central importance for determination of drug action mechanisms and as biochemical measures of drug effectiveness. During the past year, we have developed a new method for the measurement of single-strand breaks in DNA, and have explored its applicability to drug studies, as well as to problems of DNA replication and repair. The new procedure, which we call "alkaline elution analysis," has several advantages over previous methods, including precision, sensitivity, simplicity, and applicability to relatively large quantities of DNA. The method is based on our finding that cells can be lysed on membrane filters in such a way that intact DNA strands are not eluted by an alkaline solution passing thru the filter, while DNA containing single-strand breaks is rapidly eluted.

The effect of single-strand breaks was determined by subjecting cultured L1210 cells to X-ray and then measuring the fraction of the DNA that can be eluted by alkali. The dependance of X-ray dose was found to obey one-hit kinetics. It was concluded that the cell DNA exists as single-strand units that have the property that one break causes all or a large part of the strand to become elutable by alkali. From the one-hit dose, it was estimated that the X-ray target-size of the single-strand DNA units is of the order of 2×10^9 daltons. This size is in agreement with estimates of DNA single-strand lengths in mammalian cells, determined by other methods.

The replication of these large DNA single-strand units was studied by pulse-labeling and by continuous-labeling experiments. From these studies it was concluded that (1) DNA is first synthesized in the form of incomplete single-strands that are elutable by alkali, (2) these incomplete strands are are gradually converted to complete strands that are not elutable by alkali, and (3) the strands become non-elutable at the same time that their replication is completed. The time required for the replication of a single-strand DNA unit in log-phase L1210 cells was 2.8 hours. This is a substantial part of an S-phase and suggests that the time scale covers the time required for the synthesis of entire chromosomes.

With these findings as a basis, studies were initiated on the effects of anti-tumor agents on cultured L1210 cells. The effects that can be studied include (1) production of DNA breaks, (2) repair of DNA breaks, and (3) effects on replication events involved in the production of large DNA single-strand units.

Of approximately 15 agents that have so far been examined for production of DNA strand breaks, only bleomycin has been found to produce large effects. Lesser effects have been noted with several other agents, the significance of which is under study. In the case of bleomycin, the production of DNA breaks is sufficiently sensitive to be potentially applicable as a biochemical measure of drug effect. This measurement may also be of value in detecting synergism in drug combinations involving bleomycin.

Of the compounds that have so far been examined for inhibition of repair of DNA breaks, the largest effects were noted with cyclohexylisocyanate, a breakdown product of CCNU. CCNU itself had less effect.

DNA-intercalating agents.

Intercalation is a common mode of DNA binding that has been detected for several chemotherapeutic and mutagenic agents. We have had a special interest in ellipticine, an active anti-tumor drug that closely resembles the intercalator, proflavin, in molecular shape. Proflavin and related acridines, however, lack anti-tumor activity. By physical studies, it was shown that ellipticine does indeed intercalate and that the effects on DNA conformation are quantitatively similar to those of proflavin. Measurement of the binding isotherm of ellipticine to helical DNA, however, disclosed that the drug binds to DNA much more strongly than does proflavin under physiological salt conditions. The stronger binding may be the reason that ellipticine has anti-tumor activity while acridines do not. The chemical basis for the difference in binding can be understood in terms of charge, pKa, and solubility considerations.

Several intercalating agents have been studied by the technique of electric dichroism. We plan to use this technique as a rapid means to test selected new compounds for intercalating activity.

Effects on processing of high molecular weight nuclear RNA.

We had previously found that both intercalating agents and nitrosoureas such as CCNU inhibit the normal processing of 45s nucleolar RNA. We now find evidence for a similar inhibition of processing of giant nucleoplasmic RNA molecules. In the case of nitrosoureas, the effect can be seen promptly after pulse-labeling, whereas in the case of intercalators a longer time is required, probably because intercalators also inhibit chain elongation rate. In investigating the chemical basis of nitrosourea actions, it was found that cyclohexylisocyanate, a decomposition product of CCNU, is equally active in inhibiting both nucleolar and nucleoplasmic RNA processing. Since isocyanates are known to carbamylate sensitive protein sites, the results suggest that nitrosoureas may act to carbamylate sensitive sites on proteins involved in RNA processing.

Reaction of CCNU with nuclear proteins.

Since nitrosoureas such as CCNU (3-cyclohexyl-1-β-chloroethyl-1-nitrosourea) are one of the most promising groups of anti-tumor agents currently available, it is important to determine the biochemical mechanisms of action of these

reactive compounds. These compounds are able to form two reactive moieties, one of which (the chloroethyl group) can alkylate both proteins and nucleic acids, whereas the other (an actual or potential alkylisocyanate moiety) can carbamylate protein but not nucleic acids. CCNU, labeled in either of its two reactive portions, was administered to L1210 cells growing in culture, and nuclear proteins were fractionated to determine which, if any, protein species are selectively attacked. Two types of binding were found: (1) a firm, acid-stable binding, predominantly to histones, and (2) a previously unreported acid-labile binding, possibly to tyrosine or certain other amino acid residues. The acid-stable binding was found to occur almost exclusively to the lysine-rich f1 histone. The same result was obtained with both reactive halves of the CCNU molecule. The nature of the acid-labile binding will require further study. A CCNU-resistant culture line of L1210 cells has been obtained and will be studied to determine whether resistance to the drug may involve changes in nuclear protein binding.

Serial No. NCI-6103
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of Chemotherapeutic Agents on L1210 Cell Nuclear Proteins

Previous Serial Number: Same

Principal Investigators: Vincent H. Bono, Jr., Paul V. Woolley, and Robert Dion

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3
Professional:	3
Other:	0

Project Description:

Objectives:

This project is concerned with the development of techniques for the analysis of drug effects on cell nuclear proteins and with their application to drugs having both primary and secondary effects on synthesis of function of nuclear proteins.

Methods Employed:

L1210 cells were grown in suspension cultures and cloning efficiencies determined by growth of colonies in soft agar. Proteins were labeled by allowing growing cells to incorporate tritium labeled valine. Cell nuclei were isolated by homogenization in a non-ionic detergent and centrifuged through sucrose. Nuclear proteins and DNA were dissociated in high NaCl-urea and separated by ultracentrifugation. Isotopes were measured by standard methods of liquid scintillation counting. Cell DNA content was determined on formalin fixed cells which were stained with benzoflavine using a Feulgen reaction and analyzed as described under major findings.

Major Findings:

1) Effects of CCNU on nuclear proteins.

Studies on the effects of CCNU on the nuclear proteins of the L1210 leukemia

cell were continued. Over the range of 10^{-6} to $2x10^{-5}$M CCNU, the rate of cell proliferation drops from normal to zero, and viability (trypan blue exclusion and colony forming ability) drops from normal to survivals of less than 0.1% in one hour.

The uptake of the cyclohexyl moiety of CCNU by whole cells and by the cell nucleus rises steeply over this same concentration range (10^{-6} to $2x10^{-5}$M). By contrast, the cell uptake of the chloroethyl moiety of CCNU was undetectable at $1x10^{-5}$M and was only 10% of the cyclohexyl uptake at higher concentrations.

The cyclohexyl moiety of CCNU found in the cell nucleus is bound to nuclear protein with two kinds of bonds. The major type (95%) is highly pH dependent, being stable below pH 6 and unstable above pH 8. This may represent cyclohexyl carbamoyl derivatives of tyrosine or other amino acids containing hydroxyl, sulfhydryl or secondary amine grouping. The remaining 5% of the cyclohexyl moiety is stable in this pH range and may represent the cyclohexyl carbamoyl derivative of lysine.

Ion exchange chromatography of CCNU labeled nuclear protein which had been treated to remove the mild-alkali labile cyclohexyl moiety, showed that the stably bound cyclohexyl moiety is selectively bound to the very lysine rich F1 histone fraction. Although the remaining histone proteins have a significant lysine content, they contained very little cyclohexyl bound protein. This suggests that the reaction with the F1 histone involves factors other than lysine content, such as steric accessibility. Preliminary studies on the chloroethyl moiety of CCNU shows that at high concentrations (10^{-4}M) there is also a selectivity of interaction with the F1 histone fraction.

2) Characterization of an L1210 cell line resistant to BCNU in cell culture.

A BCNU resistant L1210 cell tumor (L1210/BCNU-SRI) maintained in ascites from in DBA_2 mice, was obtained from Southern Research Institute. A line of these cells was established in cell culture by direct explant in RPMI 1630 media containing 20% fetal calf serum. These cells grow in suspension culture with a doubling time of 14 hours.

This "BCNU resistant" cell culture line was capable of producing an ascites tumor when injected back into recipient BDF_1 mice (L1210/BCNU-TC). The L1210/BCNU-TC tumor and the original L1210/BCNU-SRI tumors were found to be equally resistant to BCNU therapy.

A clone of the cell culture line which survived 10^{-4}M BCNU for 10 minutes was selected by soft agar colony growth methods. From this a subclone was selected which survived 10^{-4}M BCNU for 30 minutes (L1210/BCNU-57b). The survival of L1210/BCNU-57b was 64% after exposure to $2x10^{-5}$M BCNU for 1 hour compared to 1% for the standard L1210 cell line. Continuous exposure to $1x10^{-5}$M BCNU reduced proliferation rate of both lines to 50% of control but the rate of the sensitive line fell to zero after two days of growth while the resistant line continued to proliferate at a 50% rate for 6 days. The basis for this resistance to BCNU warrants further investigation.

3) Isolation and characterization of a line of L1210 cells which grow as a monolayer (L1210-M).

It was noted that when suspension cultures of L1210 cells are grown in plastic Falcon bottles an occasional cell attaches to the surface. By replacing the medium in one of these bottles every few days over a one month period a colony of monolayer cells was isolated, (L1210-M). These cells are "epitheloid" in appearance. Electron microscopy showed the presence of intracisternal type A particles and surface budding type C particles in numbers similar to those present in the parent suspension culture. Both L1210-M and the parent culture have soft agar cloning efficiencies near 100%.

The growth characteristics of L1210-M indicate that as the colonies grow in size increasing numbers of cells are released into suspension culture. There is no evidence of "piling up" of the cells that remain fixed to the surface. Cells that are released are capable of producing monolayer colonies. Raising the medium calcium from 0.033mM to 1.33mM, adding 10^{-5} dibutyryl cyclic AMP or adding 10^{-4} theophylline had no effects on these growth characteristics. Lowering the fetal calf serum from 20% to 5% resulted in failure of the cells to either fix to the surface or grow in suspension.

Both the monolayer L1210-M and the parent L1210 suspension cultures produced tumors in DBA_2 mice, with a dose of 10^6 cells producing a similar median survival time of 17 to 20 days.

4) A study of conditions that affect the cell cycle kinetics of proliferation and the survival of L1210 cells in culture.

A study of the growth characteristics of L1210 cells was conducted in the expectation that conditions could be found for preparing populations of L1210 cells with a uniform cell cycle phase. Three methods were evaluated (a) growth to stationary stage followed by dilution into fresh medium, (b) sequential exposure to thymidine and (c) exposure to hydroxyurea either in log phase or after dilution from stationary stage.

(a) Growth to stationary stage. When grown in RPMI 1630 medium with 5 to 20% FCS, L1210 cells grow to maximum numbers of 2 to $3x10^6$/ml, the final value increasing with increasing FCS content. Using flow monofluorimetry of fluorescent-Feulgen stained cells the fraction of cells in the various phases of the cell cycle were determined. In log phase, 45% of the cells are in G1 phase. This value increases to 70% for cell populations in stationary stage and is not significantly affected by the concentration of fetal calf serum. Diluting the cells to one half of the stationary stage number in fresh medium and allowing growth back to stationary stage for several cycles failed to increase the fraction of cells in G1 to above 70%.

As cells grow in culture, the soft agar cloning efficiency drops from 80% in log phase to 40% in stationary stage. This appears not to be due to the death of cells since after dilution in fresh medium, the cloning efficiency rises to 60% over a 12 hour period (during which there is a lag in the

resumption of DNA synthesis).

(b) Sequential exposure to thymidine. Exposure of mid-log phase L1210 cells to 5mM thymidine for two 12 hour periods separated by a 12 hour period in thymidine free medium produced a population of cells which were essentially all in S-phase as determined by flow monofluorometry. However, this population could hardly be considered uniform since the cells were distributed from early to late S-phase.

(c) Exposure to hydroxyurea. Exposure of mid-log phase L1210 cells to 0.5mM hydroxyurea results in decrease in cell survival to 50% over a two hour period, followed by no further cell death over the next 6 hours. Stationary stage cells, by contrast, do not show a decrease in cell survival when exposed to hydroxyurea. When stationary stage cells (70% in G1 by flow monofluorometry) are diluted in fresh medium in the presence of 0.5mM hydroxyurea for 12 hrs., the cloning efficiency rises (as was previously seen in the absence of hydroxyurea) and by flow monofluorometry 80% of the cells appear to be in early S-phase. This represents the most uniform population of L1210 cells that we have been able to produce.

The effect of the rate of inhibition of DNA synthesis by hydroxyurea on L1210 cell survival was determined. When hydroxyurea was added incrementally so as to reduce DNA synthesis linearly over a 5 hour period to a final value of 4% of control, there was no cell death. This is in striking contrast to the 50% cell death after 2 hours that occurs when the rate of DNA synthesis is reduced rapidly to 3% of control.

Since hydroxyurea is not lethal to stationary stage cells, where DNA synthesis rates are very low, and not lethal if DNA synthesis is reduced slowly, it may be that an important factor in cell death is the rate at which DNA synthesis is reduced rather than interruption of the process per se.

5) Development of cell DNA content distribution analytic methodology and application to the effects of chemotherapeutic agents on cell cycle kinetics.

The DNA content of a cell can be used to identify a cell as being in one of the several phases of the proliferative cell cycle. In a log phase growing population the relative frequency of cells having a specified DNA content is determined by the relative time lengths of G1, S and G2. Any factor that influences these relative time lengths will alter the DNA content distribution (DCD) frequency in a predictable way.

A commerically available flow monofluorometer (BioPhysics Systems Inc.) was used to measure the fluorescence intensity (irradiated with a 488 nm laser) of cells after quantitatively labeling their DNA with a fluorescent marker using fluorescent Feulgen staining techniques. The signal was analysed and stored in a multichannel analyser (Nuclear Data Inc.) to give a frequency distribution pattern. This DCD data was then processed by computer to allow comparisons of the changes in the DCD curves induced by various perturbations,

including chemotherapeutic drugs.

The major findings to date are that as L1210 cells grow in log phase there is no change in their DCD pattern but when they enter stationary phase the fraction of cells in G1 rises from 45% to 70% but no higher. L1210 cells were exposed to several concentrations of BCNU, HN_2, hydroxyurea and 5-fluorouracil and samples taken at 3 hr, 6 hr and 24 hr for DCD analysis. Drug concentrations that reduced proliferation rates by only 10 to 20% had no major effects on the DCD patterns. Drug concentrations that reduced proliferation rates by 50% had the most interpretable effects. Each of the four drugs altered the DCD curves in a different way: (1) BCNU caused an accumulation of cells in G2 with no decrease in cells in G1 or S-phase, (2) HN_2 caused an accumulation of cells in G2 with a major decrease in cells in G1 and, to a lesser extent, S phase (3) hydroxyurea produced an accumulation of cells in G1 and early S-phase (4) 5-fluorouracil produced a decrease in cells in G1 and a major accumulation of cells in mid-S-phase. At drug concentrations that stopped proliferation promptly the major difference among the drug effects was in the appearance of cells with less than a G1 DNA content: (1) BCNU produced a small pre-G1 population with no major change in the DCD curve. (2) HN_2 produced a major pre-G1 population with evidence of a general decrease in the DNA content of all cells. (3) Hydroxyurea produced a pattern similar to that produced by HN_2 (4) 5-fluorouracil produced the smallest pre-G1 population with no significant change in the DCD curve.

Proposed Course and Relevance to the Program:

(1) Studies performed this year indicate that interruption of DNA synthesis in proliferating L1210 cells does not always result in cell death. We plan to pursue analysis of this observation to (a) further define the conditions that are required and (b) determine if the relationship of DNA synthesis to histone protein synthesis plays a role in this phenomenon. The practical aspect of this line of research is that if a more complete understanding of the relationship between DNA synthesis inhibition and cell death was available we might be in a better position to modify the relative cytocidal effects on host and tumor cells. (2) Studies on the direct interaction effects of CCNU with nuclear proteins will proceed to (a) further define the character of mild alkaline labile bonds with nuclear proteins and (b) examine the characteristics of the BCNU resistant cell culture line (L1210/BCNU-57b). (3) Further develop the analysis of drug effects on the progress of cells through the proliferative cell cycle using cell DNA content distribution methodology. It is anticipated that this analysis may allow a characterization of some of the molecular pharmacologic properties of chemotherapeutically active agents, their structural analogs and cytotoxic agents of unknown chemotherapeutic activity.

Publications: None

Serial No. NCI-6104
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of Chemotherapeutic Agents on RNA Metabolism

Previous Serial Number: Same

Principal Investigators: Herbert E. Kann, Zafar Iqbal, and Kurt W. Kohn

Other Investigators: None

Cooperating Units: R. Reagan Laboratory of Toxicology, NCI (electron microscopy).

Man Years:

Total:	3.5
Professional:	2.5
Other:	1

Project Description:

Objectives:

To examine a variety of chemotherapeutic agents, known to interfere with RNA synthesis, for selective effects on the patterns of RNA synthesis and processing. This information may help to determine the mechanisms of action of these agents and to classify these agents according to the effects produced. In addition, this study aims to utilize specific drug actions to elucidate the mechanisms of RNA synthesis and processing in the cell nucleus.

Methods Employed:

1) Growth of L1210 cells in suspension culture.

2) Incorporation of ^{3}H and ^{14}C RNA precursors; scintillation counting.

3) Cell fractionation and RNA isolation.

4) RNA sedimentation in sucrose gradients.

5) Time-sharing computer.

6) Gel electrophoresis.

7) Electron microscopy.

Major Findings:

1) Effects of chemotherapeutic agents on RNA metabolism.

We have previously reported that similar effects on RNA metabolism are produced by intercalating agents (ellipticine, proflavine, ethidium) and by some nitrosourea compounds (BCNU, CCNU). These effects were: a) inhibition of the rate of incorporation of radioactive uridine into RNA, under conditions in which drug exposure precedes the addition of isotopic precursor; and b) inhibition of the processing of nucleolar RNA to its normal products, 28 and 18s ribosomal RNA. Identical results were obtained using chloroethyl isocyanate (NSC 87418) or cyclohexyl isocyanate (NSC 87419). Chloroethylamine (NSC 10871) was inactive.

During the past year we have found that these same compounds also prevent the normal processing of nucleoplasmic RNA. In the absence of drug action nucleoplasmic RNA molecules are either degraded within the nucleus or are converted to smaller messenger RNA molecules that pass into the cytoplasm. The drug effect is revealed by an excessive accumulation, during long labeling periods, of very rapidly sedimenting nucleoplasmic RNA.

Chloroethylisocyanate and cyclohexylisocyanate are particularly active inhibitors of nuclear RNA processing, yet they do not bind to any appreciable extent to nucleic acid. Since they do bind extensively to proteins we suspect that their action may be due to inactivation of RNA processing enzymes.

2) Ribonuclease sensitivity of RNA species.

This study had been initiated in response to our finding of inhibition of the processing of high molecular weight nuclear RNA by intercalating agents, which led to the hypothesis that helical regions may exist in these RNA species. These helical regions may be recognition sites for specific processing enzymes and may be blocked by intercalation. The objective was to determine whether ribonuclease reaction kinetics could be used to measure the helical content of RNA. This approach initially seemed successful in that the kinetics disclosed two components that could be interpreted as separate sensitive and resistant components of the RNA. We had shown that the slow component of the kinetics is not due to loss of enzyme activity, and it was found as expected that the degradation of the U-strand in poly A-poly U has no resistant component in the kinetics. The unexpected result was then obtained that poly A itself has two components to the kinetics. It was at first thought that this might be due to a reduced ribonuclease-sensitivity of chains shorter than a critical length. Partially digested poly A was therefore re-isolated after a suitable incubation with ribonuclease and then used as substrate in a separate ribonuclease reaction. The surprising result was that the kinetics again were biphasic. This experiment was then carried out with cell RNA species. Cell RNA was digested with ribonuclease until the fast component of the reaction was passed. The RNA was then reisolated and used in a separate ribonuclease

reaction. Again the reaction was biphasic. These results show that the two component kinetics, heretofore taken as a measure of RNA secondary structure, is instead due to an anomoly in the enzyme reaction. This phenomenon must be taken into account in studies in which ribonuclease digestion is used to isolate resistant RNA structures, and may be responsible for conflicting reports on the sizes of such structures.

Proposed Course:

No further work in this area is planned during the coming year.

Publications:

1) Herbert E. Kann, Jr., and Kurt W. Kohn: Effects of Anthramycin and Actinomycin on RNA Synthesis Patterns in L1210 Cells. J. Cellular Physiology. 79: 331, 1972.

2) Herbert E. Kann, Jr., and Kurt W. Kohn: Effects of Deoxyribonucleic Acid-Reactive Drugs on Ribonucleic Acid Synthesis in Leukemia L1210 Cells. Molecular Pharmacology. 8: 551, 1972.

Serial No. NCI-6112
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of Chemotherapeutic Agents on DNA

Previous Serial Number: Same

Principal Investigators: Kurt W. Kohn, Charles A. Friedman, Regina A. Grimek-Ewig, Zafar Iqbal, Daniel Glaubiger, and Herbert E. Kann, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	4.8
Professional:	3.3
Other:	1.5

Project Description:

Objectives:

To determine the mechanisms of interaction of chemotherapeutic drugs with DNA, and the consequences of such interactions on DNA structure and function.

Methods Employed:

1) Growth of L1210 cells in suspension culture; quantitative determination of colony-forming ability by cloning in soft agar; incorporation of radioactive precursors; X-irradiation of cells; special procedures for analysis of state of DNA in cells, described herein; preparative ultracentrifugation in density gradients.

2) Spectrophotometric determination of binding constants; analytical ultracentrifugation; viscometry; circular dichroism; electric dichroism.

Major Findings:

1) Development of alkaline elution technique.

The major accomplishment under this project during the past year has been the development and application of a new technique for the determination of the integrity of DNA single strands in cells. The impetus for the development of this technique has been its potential applicability for the detection and

measurement of early cell damage that may be related to the cytotoxic action of drugs. The objective was to devise a highly sensitive measure of DNA strand breakage that could be used as a convenient assay in studies of drug action on cells. In addition to the accomplishment of this objective, the technique was successfully applied to studies of DNA replication that permitted measurements on a larger DNA size scale than had previously been possible by biochemical methods.

The basis for the new technique was our finding that cells can be lysed on membrane filters and then treated with alkali in such a way as to selectively elute from the filters DNA that contains single-strand breaks; DNA that is incompletely replicated also was found to be selectively eluted. Most of these studies were carried out on suspension cultures of L1210 cells, labeled with ^{14}C- or ^{3}H-thymidine for various times. The cells were deposited and washed on membrane filters and then lysed on the filters with a high-salt-detergent (HSD) solution. Microscopic examination (Zeiss-Nomarski interference optics) had shown that cells mixed with this solution lose all visible structure except for occasional bodies resembling nucleoli; all membrane structures appeared to be dissolved. Washing the filters with HSD solution removed part of the cell protein but little or no cell DNA was washed thru (unless the cells had been severely damaged by drug treatment). When the filters were then slowly eluted with alkali, the cell DNA gradually came out thru the filters over a period of several hours. If the cells had been X-irradiated with moderated doses (e.g. 1000 rad), the DNA came out much faster than from control cells. The difference could in fact easily be seen by eye, since the alkaline solution dripping from the funnel became markedly "stringy", a characteristic commonly observed in solutions containing high molecular weight DNA. Since the DNA concentration was always below 2 µg/ml, the DNA single strands in the alkaline solution must be of extreme length to produce such a marked change in the gross physical properties of the solution. This was later confirmed by alkaline sucrose gradient sedimentation (v.i.). Further indication of the high sensitivity of the effect came from the observation that easily detectable effects were produced by X-ray doses as low as 100 rad, which would produce only one break per approximately $5x10^9$ daltons of DNA single strand. This sensitivity equals or exceeds the best that has been attainable by alkaline sucrose gradient sedimentation.

The next task was to make the procedure quantitatively reproducible, and applicable to measurements of the physical state of DNA in cells. This was accomplished in several steps during the year, as additional characteristics of the system emerged and led to methodologic improvements. In order to control the elution rate, the exit tubing from the filter funnel was modified so that it could be connected to a persistaltic pump leading to a fraction collector. A variety of conditions were explored with the objective of minimizing the elution of long-label DNA from control cells while maximizing the elution of broken or incomplete DNA strands. The following methodologic factors were found to have significant effects on the elution: (1) type of filter material (pore size was not critical), (2) presence of EDTA, (3) type of cation in the alkaline eluting solution, (4) OH^-

concentration, and (5) exposure to room fluorescent light during the alkaline step. The major problem was a variable elution of the control DNA. Considerable variability was found between different types of filters, sometimes even between different lots of the same type. The best type of material considering also convenience and consistancy, was polyvinylchloride (2 micron pore size was used). The presence of EDTA in the alkaline eluting solution tended to reduce the loss of control DNA. The same was true for replacement of NaOH by tetrapropylammonium hydroxide. The OH^- concentration was critical at about pH 11.5, below which little or no DNA was eluted, and above which the elution of incomplete DNA chains appeared to be complete. This is the same critical pH at which DNA double helix dissociates into single strands. The elution of control DNA, on the other hand, increased progressively as pH was increased beyond 11.5. Room fluorescent light was found to markedly increase the elution of control DNA, especially at high pH. The best results were obtained when the elution was carried out in total darkness, permitting a clear separation between elutable and non-elutable DNA.

2) Alkaline sucrose gradient sedimentation of eluted DNA.

In order to determine its size range, it was necessary to layer the eluted DNA on top of a sucrose gradient without breaking the highly sensitive DNA strands. To minimize shearing, the eluted DNA was very slowly pumped directly onto the gradient. The results showed that the eluted strands sedimented similarly to DNA from cells lysed directly on the gradient, indicating that the strands were not fragmented during the elution and collection procedures. Since little or no protein was eluted with the DNA, this procedure for the first time permits sedimentation studies of purified and unfragmented DNA strands from mammalian cells. The elution produced a partial fractionation of the DNA in that DNA size tended to increase with time during the elution. The sedimentation also tended to increase with duration of ^{3}H-thymidine pulse, reflecting the growth of replicating DNA chains.

3) X-ray sensitivity of single-strand DNA units.

Measurements of the effect of X-ray on the elution provided information about the nature of non-elutable DNA. Cells were X-irradiated at ice temperatures so as to prevent enzymatic DNA repair. With increasing X-ray dose, non-elutable DNA was converted to elutable DNA. The loss of non-elutable DNA was first-order in X-ray dose; the 1-hit dose (D_{37}) was approximately 300 rad. Quantitatively consistant data was obtained in several independent experiments, including several variations in experimental procedure. The first-order X-ray sensitivity indicates that a single X-ray event can convert a DNA strand from a non-elutable to an elutable state. The target-size of the strands, estimated from the D_{37} dose, was approximately $1.5\text{-}3 \times 10^9$ daltons.

4) Replication of single-strand DNA units.

The replication of kinetics of the large DNA single-strand units defined above was studied by pulse-chase and by continuous labeling experiments. After pulse-labeling for 10 minutes with ^{3}H-thymidine, cells were resuspended in fresh medium and analyzed at various times thereafter. The fraction of the ^{3}H-DNA that was unelutable increased linearly with time and reached 50% at about 1.5 hours. Extrapolation back to zero time, indicated that less than 10% of the ^{3}H-thymidine incorporation goes directly into non-elutable DNA. From these findings, it was possible to conclude that the DNA single-strand units, defined above on the basis of X-ray sensitivity, become non-elutable at or about the time that their replication is completed.

Continuous labeling studies showed that ^{3}H-thymidine incorporation into elutable DNA increases with time to a constant level, while incorporation into non-elutable DNA lags and then increases linearly. The results were quantitatively consistant with a kinetic model based on a simple precursor-product relation between elutable and non-elutable DNA. Experiments of this type will permit measurement of the time required for replication of a DNA single-strand unit, and of the average amount of elutable DNA per cell. In exponentially growing L1210 cells, the values found were 2.8 hours for the replication time and 8.4% of a G1-cell-equivalent for the average amount of elutable DNA. The replication time of a DNA single-strand unit is about 40% of an S-phase period, and therefore is on a time scale that may encompase the replication of an entire chromosome.

5) DNA repair.

The expected DNA repair after X-irradiation of cells was easily measurable by the alkaline elution technique. Several anti-tumor drugs were examined for possible inhibition of the repair of DNA single-strand breaks. No anti-tumor drugs having a high degree of activity have so far been detected.

Because of our previous findings that nitrosoureas inhibit the processing of high molecular weight nuclear RNA, we paid special attention to this type of agent as potential inhibitors of DNA repair. Partial inhibition of the repair of DNA single-strand breaks was indeed detected with BCNU. But the concentrations required were relatively high and bordered on the concentrations which themselves produced DNA breaks. However, chloroethylisocyanate, a break-down product of BCNU, caused distinct inhibition at lower concentrations. These compounds will require further investigation.

Studies are also in progress to determine the effects of drugs on the ability of cells to repair DNA and to survive after UV irradiation.

6) DNA breaks in drug-treated cells.

We are examining a variety of anti-tumor drugs for production of DNA breaks in cells. The objective here is not solely to determine which drugs produce breaks as a primary action, but also to explore the possibility that DNA breakage, even if it is a secondary effect, may in certain cases be a measure of cytotoxicity that could perhaps be applied as an *in vitro* test for drug

sensitivity. The alkaline elution methodology lends itself to this objective because the procedure is simple enough to be adapted to routine assay and also can be adapted to the measurement of non-radioactive DNA. Of the compounds that have so far been examined, only bleomycin has produced large breakage effects. This effect of bleomycin is consistant with the known DNA breakage effect of this compound in chemical systems. Several agents were found to produce lesser effects, the possible significance of which is now being investigated by comparing effects on DNA with effect on colony-forming ability.

7) Binding of ellipticine to DNA.

Because of the similarity in shape of the ellipticine molecule to proflavin, we have suspected that ellipticine, like proflavin, is an intercalating agent. We have now confirmed this by physical methods. The effects of the two compounds on the viscosity and sedimentation of DNA was found to be quantitatively similar for equal extents of binding. Thus, the two compounds produce equal extents of lengthing and stiffening of the DNA helix. Independent confirmation was obtained by electric dichroism measurements which showed that the chromophore of bound ellipticine is paralell to the DNA bases.

Since both compounds exhibit similar physical interaction with DNA, and as we have previously shown, both affect RNA synthesis in a similar way, the question arises why one has anti-tumor activity while the other has little or none. Although we have no adequate answer for this question, we have found that ellipticine has a much greater binding constant for DNA than does proflavin. These measurements were carried out by spectrophotometry in 0.06M $NaPO_4$ buffer. The ionic strength of this buffer is in the physiological range, and is sufficiently high to reduce the affinity of proflavin for DNA. Special care was required in carrying out these measurements, because ellipticine has a very low solubility at neutral pH and a high tendancy to adsorb to glass. The spectral measurements were then successfully carried out under conditions under which Beer's law was obeyed. The results showed an association constant of $3 \times 10^7 M^{-1}$ for native DNA and $4 \times 10^5 M^{-1}$ for denatured DNA. The much higher affinity for native than for denatured DNA must be due to a strong preference for the intercalative mode of binding.

Ellipticine can be protonated and has a pKa of 5.7. The molecule therefore acquires a positive charge at low pH. It seems likely that bound ellipticine becomes protonated and that the resulting positive charges helps to stabilize the complex. This idea has supported absorption and circular dichroism spectra.

8) Electric dichroism of drug-DNA complexes.

These studies were initiated last year in collaboration with Dr. Elliott Charney, NIAMDD. The measurements with anthramycin made possible an estimate of the angle between the anthramycin chromaphore and plane of the DNA bases. The angle was estimated to be about 45°, thus excluding intercalation as the the mode of binding. Measurements on ethidium, daunomycin, and ellipticine

showed that the plane of these molecules bound to DNA is parallel to the plane of the bases, as expected for intercalating agents.

Proposed Course:

1) Investigate mechanism of rentention of cell DNA on filters during alkaline elution: is the retention due simply to the large size of the DNA molecules, or is it due to anomalies of structure or attachment of the DNA.

2) Investigate mechanism of the release of DNA due to exposure to light in the presence of alkali: is the photochemically sensitive absorber DNA or some other substance.

3) Continue studies aimed at improving sensitivity and versatility of the alkaline elution technique. Determine whether the technique is applicable to tissues from whole animals.

4) Repeat studies of X-ray sensitivity and replication using improved technique. Compare various cell types.

5) By means of alkaline elution technique, study effects of drugs in cells, with respect to (a) production of DNA breaks, (b) inhibition of repair of breaks, (c) DNA replication. Determine how any observed effects relate to survival of colony-forming ability.

6) Explore the possibility that the above measures may be used to predict the sensitivity of cells to particular drugs.

7) Use electric dichroism to determine intercalating ability of selected compounds produced through the synthesis program.

Publications:

1) Kurt W. Kohn and Regina A. Grimek-Ewig: New Approach to the study of DNA Single-Strand Interruptions in Cells:Alkaline Elution of DNA Trapped on Filters. Cancer Research (in press).

SUMMARY REPORT

LABORATORY OF TOXICOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1972 - June 30, 1973

I. Introduction

Ever since its inception four years ago, the Laboratory of Toxicology has retained responsibility for supervision of studies of large-animal-toxicology of antineoplastic agents. This important role is actually accomplished by the contract mechanism, but it is the responsibility of the Laboratory of Toxicology to direct these studies, summarize the information, and aid in the submission of an INDA to the Food and Drug Administration prior to initiation of Phase I Clinical Trials. Thus the position of the Laboratory of Toxicology at Stage III of the Linear Array represents a very crucial junction in the drug development program. If the studies from this area are not promptly completed, the introduction of new antineoplastic agents into general medical practice can be delayed for several years. Two major developments have occurred during the past year to improve the quality of the toxicology studies and to attempt to accelerate the rate at which these studies are completed. The two events are: 1) the improvement of the prowess of the in-house staff of the Laboratory of Toxicology in basic research; and 2) the granting of permission by Division of Cancer Treatment Program Staff to develop and initiate a prime contract, which during the next fiscal year, will aid in the more rapid delivery of the completed protocol studies. Each of these items will be discussed in further detail.

A new level of excellence in basic research was sought on the part of the Laboratory of Toxicology Senior Staff. The actual number of contracts supervised by each Senior member of the Staff has been distributed more uniformly. This gives each Project Officer fewer contractors to supervise and thus allows for a greater concentrated effort in providing guidance where necessary. Of course this change also was designed to increase the amount of time that the senior staff members would have for personal research. The obvious point here is that supervision of contractors should be done by members of the scientific staff who have considerable first hand knowledge about research involving the use of antineoplastic agents. It is strongly felt that if these staff members are not highly respected members of the scientific community their advice to contractors would be hollow indeed. Such a posture also is necessary to allow the internal staff to expand its capability of responding quickly to a new kind of toxicity or new kind of side effect which may be uncovered by the contractors. The presence of a competent internal staff has increased somewhat the awareness level of such problems at very nascent stages of development. This

awareness also allows for the mobilization of both internal and external programs to focus on this newly discovered type of toxicity. Problems of this nature which already have been faced include cardiotoxicity from adriamycin, pulmonary fibrosis from bleomycin, and ototoxicity from platinum compounds. It is only by a combination of quick recognition of these problems and the ability to recruit the best resources, that such problems can be on the path to solution by the time clinical trials are initiated. While the degree of emphasis on basic research prowess represents a turn up a new road as far as the Laboratory is concerned it must be admitted in this first year that we have not travelled very far. While there are a few signs that some progress is being made in this area, perhaps it is too early to judge. In summary then, the contract responsibility has not diminished, but rather, improved internal research has been added to this responsibility.

II. Internal Research Programs

All of the independent projects of the Senior Staff Members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only by understanding these actions that we will be able to advise clinicians intelligently as to what toxicity may occur and if it does occur, what steps maybe taken to reverse this toxicity.

A. Biochemistry Unit

The Biochemistry Unit is engaged in six major projects. Four of these, as in previous years, are concerned primarily with the oncolytic enzyme L-asparaginase. In two of these studies of tumoral L-asparagine synthetase, the partial purification of this enzyme is being attempted. In a related project, various properties and inhibitors of this enzyme are being examined. The purpose of studies of this synthetase is to determine an efficient means for inactivating the enzyme in order to enhance the antineoplastic activity of L-asparaginase. Purification of L-asparagine synthetase has involved extraction, heat treatment, ammonium sulfate precipitation, DEAE chromatography, Sephedex G-100 gel filtration, and preparative gel electrophoresis. These various steps have yielded an enzyme which is about 120 fold purer than the starting material. The systematic search for inhibitors of L-asparagine synthetase was prompted by desire to enlarge the therapeutic scope of L-asparaginase by either preventing the emergence of resistance via induction of L-asparagine synthetase, or by converting formerly resistant neoplasms to sensitive variants by cancelling their mechanism of resistance. The most potent inhibitor found this year is Thiomersal, which causes about a 99% inhibition at 1×10^{-3}M. Significant inhibition, greater than 50%, also occurred from 5-Isoxazole-acetic acid, α-amino-3-chloro-4,5-dihydro and Japanese Neocarzinostatin. Substituted maleamic acids, monomaleimides, dimaleimides and chlorosuccinamic acids were synthesized and tested for their ability to inhibit L-asparagine synthetase. Other classes of inhibitors which have been tested are mucochloric and mucobromic acids, as well as DON, DONV, and CONV. Also studies have been conducted where L-asparaginase has been coupled to Dacron vascular prostheses with retention of enzymatic activity. During this past year studies of the biochemical and immunological properties of such prostheses have been continued

and largely concluded. In the fourth research area involving L-asparaginase, purification of L-asparaginase from the agouti has been initiated to eventually characterize the enzyme (a glutaminase-free-hydrolase) from this source.

The other two antineoplastic agents receiving attention from the Biochemistry Unit are Azotomycin and Streptozotocin. In studying the mechanism of toxicity produced by Azotomycin, it was noted that this agent caused convulsions in mice. Histopathologic evidence of the convulsions was observed by noting that there was marked vacuolar degeneration of portions of the cerebellum of treated mice. Animals showing the convulsions demonstrated EEG abnormalities typical of the epileptic pattern. The lesion was further characterized biochemically by demonstrating that L-glutamate, a proposed neurotransmitter, had accumulated in brains of treated animals. Thus the study followed a classic progression commencing with observations of convulsions, demonstrating EEG abnormalities, histopathologic changes and culminating in the demonstration that the biochemical basis for the lesion involved a build-up of a probable neurotransmitter. Part of the biochemical mechanism of action of Streptozotocin apparently involves alkylation of DNA. Therefore studies have been undertaken to determine whether nicotinamide, a vitamin which reverses the diabetic effect of Streptozotocin, would also diminish alkylation of DNA. Hence this is a study in which a biochemical mechanism of toxicity is being undertaken with a further goal of applying a possible molecular antidote to the toxicity.

Hence, during the present year the Biochemistry Unit has provided the research leadership for the Laboratory of Toxicology. While the primary efforts of this group have focused on the actions of the oncolytic enzyme, L-asparaginase, the very basic nature of their research in the realm of biochemistry is very important to the program. By maintaining such a unit in sharp focus to problems of enzymatic and/or biochemical nature, the program has an invaluable resource.

B. Comparative Pathology Unit

This unit has been instrumental in finalizing yet another revised protocol for the conduct of toxicological studies of potential antineoplastic agents. This further revision of the protocol highlights the dynamic nature of the needs of the Laboratory of Toxicology. It is felt that the present protocol represents the most detailed document available. This version of the protocol will become the primary document to be employed by the Prime Contractor. Comparative pathology personnel have built into the protocol a method for tabulating and eventually computerizing quantitative relationships of the toxicity of antineoplastic agents in dogs and monkeys. Another primary effort this past year has been in extending the analysis of such data to include not only the animal studies but also the results from autopsy studies done at the Clinical Center. The primary research efforts of this group have been in elucidating the pathologic effects of two antineoplastic agents. In one study the rabbit was employed in attempts to show how L-asparaginase induces hypocalcemic tetany. The results of this work showed a remarkably specific effect of L-asparaginase on the parathyroid gland of this species where degranulation and autophagy were observed. Preliminary experiments have been conducted in an attempt to

evaluate the possible effect of adriamycin on mineral metabolism in the rabbit heart. Because of the dose-limiting cardiotoxicity of this agent such studies are of great importance in attempting to define the mechanism by which adriamycin-type agents cause this toxicity.

C. Drug Interactions Unit

The newest group of the Laboratory, the Drug Interactions Unit has not been in existence for one year. This unit was created because of the obvious importance of studying drug interactions in a field which commonly employs multiple drug combinations. The group has shown capabilities of studying interactions in the following areas: enchancement or inhibition of microsomal drug metabolism, competition and/or displacement of one agent by another by plasma protein binding, and studies of biliary and urinary excretion of anti-neoplastic agents and model compounds. In one study, for example, the availability of three intravenous dose formulations of CCNU suggested that these three formulations should be evaluated for possible differences in tissue distribution. The results of this study of three vehicles, side by side, in a single species are not available. This group has completed an extensive study in the rabbit which involved fifteen tissues and three body fluids which were analyzed at 10 minutes, 1, 4, 12, and 24 hours after intravenous administration. With this study they have established that there are no major differences in the distribution of the drug which are dependent on the vehicle. These results suggest that the ultimate decision as to which vehicle to use should be determined by other factors such as ease of handling of the dose formulation and/or differences in vehicle toxicity. Another project initiated by this unit involves an effect at the microsomal level which, like the hematologic toxicity of BCNU, CCNU and Methyl CCNU is incredibly delayed. Previous studies by others have shown that there is up to a 90% reduction in the capacity of the liver to metabolize drugs as long as three weeks after a single dose of one of these agents. Detailed studies are being undertaken to elucidate this unique kind of toxicity which is manifested primarily in hematologic and microsomal toxicity. Such studies of these and other agents are very important since they point out some hazards associated with the use of these agents in patients who can little afford to be treated with combinations of drugs which might have greatly increased toxicity because of compromised ability of the liver to catabolize other drugs which might be used in adjunct therapy. This research group has continued studies in the area of species differences in drug metabolism. These studies are of importance since one always seeks to employ animals which metabolize drugs in a manner similar to man. If one cannot find a species which does so, it is advantageous to sometimes employ species with impaired ability to metabolize drugs as such an effect would exacerbate a toxic sign. The manifestation of such toxicity in an animal would suggest to a clinician that a susceptible individual might show symptomatic toxicity of this kind.

D. Collaborative Efforts With Other NCI Units

The Laboratory of Toxicology has taken steps to increase the interaction of other segments of the linear array not only at formal meetings of the Decision

Network and Operating Committee but also by joint seminars and informal meetings on a day-to-day basis. In particular, because of the proximity of these groups to the Laboratory of Toxicology in the linear array, new levels of cooperation have been achieved with the dose formulation and clinical trials areas. The pressing need to develop vehicles to solubilize lipoidal antineoplastic agents lead the Laboratory of Toxicology to encourage dose formulation personnel to explore a variety of such vehicles. We have given support, by preliminary testing in animals of such vehicles and thus have provided this section with very rapid assessment of the biologic effects of these solutions. One "spin off" of such collaborative efforts has already resulted in a collaborative publication which NIMH has laureled since these efforts have provided them with a parenteral formulation for tetrahydrocannabinol, the active ingredient of marijuana. The application of the vehicle emulphor in this situation followed from its utilization with other lipid soluble antineoplastic agents. Collaboration with the clinical trials group has resulted from a common interest of the Laboratory of Toxicology and this group in several areas, most particularly in the area of combination chemotherapy and drug interactions. Since the clinical trials area uses the protocol toxicology studies as part of the INDA, day-to-day questions from them regarding these studies have been promptly answered in attempts to accelerate the process by which new drugs are introduced into clinical trials. Other members of the Laboratory of Toxicology have collaborated with the Membrane Transport Section, OADET, in the study of the distribution of CCNU in three different vehicles, propylene glycol:ethanol, emulphor, and intralipid. The expertise of this group in the area of distribution studies and computerization of such studies has been very helpful.

E. Collaborative Efforts With Other NIH Units

As far as the scientific aspects of the performance of the Laboratory of Toxicology in the past year, clearly the most innovative and promising area has been in this new program of research of computer-assisted methods for quantification of structure-activity relationships. The major objective has been the application of digital computers to assist in quantifying structure-activity relationships of drugs. While other segments of the drug development program do make extensive use of computers in the acquisition, storage and retrieval of information on antineoplastic drugs, this new approach is designed to use computers as learning and predictive devices. Some of the studies were based on the fact that the ultimate mechanism by which a drug exerts its pharmacologic or toxicologic action is by the interaction of drug molecules with macromolecular receptors in the body. It is felt that instead of studying classical physical parameters such as lipid solubility, investigation of the mass spectral fragmentation pattern may be a better indicator of which portions of the drug molecule are necessary for a given biologic activity. The initial studies in this area, to be published shortly in Science, have shown that this method can correctly predict at least 80% of the time the pharmacologic activity of a molecule by consideration of its mass spectrum. This computer learning process is being applied to two difficult program problems; one is the investigation of the mass spectra of a series of analogs of established antineoplastic agents to attempt to predict which compounds might possess the best antineoplastic activity. Two classes of agents being studied in this

manner include the nitrosoureas and imidazole carboxamides. A second area in which computer assisted techniques are being employed will establish a computer based predictor system for the metabolic pathways that a drug might undergo. It is hoped that this method will aid by suggesting which of dozens of possible metabolites should be sought first by the analytic chemist. Both of these computer originated studies should greatly aid the drug development program by providing clues to the synthetic chemist as to which structural features are associated with the best antineoplastic activity and further to identify likely metabolic pathways for these agents. The present procedure for accomplishing both of these goals are laborious, time consuming and expensive. Although no substitute for the biologic work, the computer should greatly aid in narrowing down an almost infinite scope of possibilities to a reasonable number of items which the chemist or biochemical pharmacologist can follow. This project has allowed the Laboratory of Toxicology personnel a unique opportunity to collaborate with chemists, huristics experts, computer scientists, mathematicians, and members of DR&D Branch. The non-NCI members of this group have been most interested in applying their unique skills to the very practical problems of NCI discussed above.

III. Contract Programs

A. The Prime Contract

The Laboratory of Toxicology is pioneering in the development of the prime contract concept in the Division of Cancer Treatment. The concept, coupled with the incentive award approach, is clearly innovative for this segment of the program and may have applicability to other areas of the linear array of the drug development program. The efforts of the Laboratory of Toxicology have been rewarded by excellent cooperation from the contract specialists of the Research Contracts Branch of NCI. This team effort has provided a unique opportunity for our scientists to learn about management as well as to provide an opportunity for management to understand some of the scientific aspects of our problems. The motivation behind consideration of this concept of contracting is 1) the need to develop unified management control of the scientific aspects of the protocol toxicology studies; and 2) the growth trend of this segment of the linear array caused by the influx of compounds from the screening program and non-government sources. The primary objective of the prime contract will be to have the day-to-day supervision of the actual toxicology studies assumed by such a contractor and have delivered to the Laboratory of Toxicology the data in a format suitable for submission to the Food and Drug Administration as a supporting document for the Investigational New Drug Application. A second objective of the prime contract will be to develop a "quick reaction" capability for high priority drugs or analogues of oncolytic agents already in clinical use. Another important objective will be to identify and code toxicity data that will allow the Laboratory of Toxicology to improve the predictability of toxicity.

B. Present Contract Program

The present contractors for the Laboratory of Toxicology have been encouraged to focus their efforts toward completing protocol studies on those agents that

have been under study for a considerable length of time. Of course, the need for prompt completion of newly assigned drugs also has been impressed on the contractors. These dual admonitions have resulted in the completion of protocol-type toxicology on eleven (11) agents this year. INDA's have been submitted or are in the process of being submitted for most of these agents. This past year has seen the completion of nine (9) special toxicity studies while we ended the year with twelve (12) ongoing protocol studies. The detectable level of effort of the contractors, especially in the area of completion of protocol studies has increased dramatically this fiscal year. Another area in which some of our contractors have been helpful is in developing "mini-models" for screening for specific organ toxicity. Some success can be claimed in the following areas: 1) the rabbit model for adriamycin cardiotoxicity; 2) guinea-pig model for platinum-induced ototoxicity; 3) mouse screen for platinum-induced renal toxicity; 4) monkey model for platinum ototoxicity; and 5) the dog model for bleomycin induced pulmonary fibrosis.

Serial No. NCI-3518
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: L-Asparagine: Synthesis, Biological Role and Hydrolysis

Previous Serial Number: Same

Principal Investigator: David A. Cooney

Other Investigators: Hiremagalur Jayaram, James Althaus, Harry Milman, Ruth Davis

Cooperating Units: Howard Weetall, Lee Hersch, Corning Glass Company, Corning, New York
Ulrich Schaeppi, Mason Research Institute, Worcester, Mass.
John Driscoll, Division of Cancer Treatment, National Cancer Institute

Man Years:

Total:	3
Professional:	1 1/2
Others:	1 1/2

Objectives:

It is the objective of the present research to characterize L-asparagine metabolism and L-asparagine synthetase from neoplastic and normal tissue, and to determine the most efficient means of inactivating that enzyme. It is hoped that an active chemotherapeutic agent will result from such an approach.

Principal Findings:

I. Studies with L-Asparagine Synthetase: Partial Purification of the Tumoral Enzyme

During the past year, substantial progress has been made in the purification of L-asparagine synethetase from mouse L5178Y leukemic cells rendered resistant to L-asparaginase by repeated injections with subcurative doses of L-asparaginase. The following procedures were investigated as steps for the purification: heat treatment, ammonium sulfate precipitation, ethanol treatment, acetone fractionation, isoelectric precipitation, chromatographic

techniques utilizing DEAE cellulose, affinity chromatography, agarose cross-linked with L-asparagine, L-aspartic acid or L-glutamine, DEAE-Sephadex, CM-cellulose, CM-Sephadex, and different grades of Sephadex gels for filtration and gel electrophoresis. We adopted the following steps for the purification of L5178Y/AR soluble tumoral L-asparagine synthetase.

Tumor Extract: L5178Y/AR tumor cells were collected from 10-12 day old, subcutaneously transplanted tumors in BDF_1 mice and homogenized in 10 volumes of 0.1 Tris HCl buffer (pH 7.6) containing 5mM EDTA and 1mM dithiothreitol. The homogenate was centrifuged at approximately 30,000 x g for 1 hour. The supernatant of the tumor extract after centrifugation was collected and processed as follows:

Heat Treatment: To the tumor extract after centrifugation was added 0.001 M ATP, $MgCl_2$, L-glutamine and ammonium chloride to protect the enzyme from denaturation; the extract so enriched was then heated at 50°C for 10 minutes, immediately chilled on ice and centrifuged at approximately 30,000 x g for 1 hour. This step separates heat-inactivated proteins, nucleic acids and any residual particulate enzymes from the active enzyme protein which remains in the supernatant. There was about 30% loss of enzyme activity with a 1-2 fold purification.

Ammonium Sulfate Precipitation: The heated centrifuged extract was brought to 30% saturation with ammonium sulfate, and the precipitated proteins were removed after centrifugation at 20,000 x g for 30 minutes. The supernatant was brought to 50% saturation with ammonium sulfate and the precipitate was collected after centrifugation at 20,000 x g for 30 minutes. The 30 to 50% ammonium sulfate precipitate was resuspended in 0.001 M Tris HCl buffer (pH 7.6) containing 0.001 M $MgCl_2$, L-glutamine and ammonium chloride and 10% glycerol (hereafter called J-Buffer) and dialyzed at 5° against the same buffer. About 10 fold purification was achieved at this step. There was no further loss of enzyme activity.

DEAE Chromatography: Washed DEAE cellulose suspended in water was poured into glass columns and equilibrated with J-Buffer. The dialyzed ammonium sulfate fraction was loaded onto the DEAE column and washed first with J-Buffer and then with 0.1 M Tris HCl buffer (pH 7.6) containing 0.001 M $MgCl_2$, L-glutamine, ammonium sulfate and 10% glycerol. The active enzyme protein was eluted from the column using a linear gradient (0.1 to 0.5 M Tris HCl, pH 7.6) containing 0.001 M $MgCl_2$, L-glutamine, ammonium chloride and 10% glycerol; the active protein eluted at 0.22M Tris HCl buffer concentration. There was about 50% recovery and 100 fold purification at this stage.

Ammonium Sulfate Precipitation: Active fractions from the DEAE column were pooled and an equal volume of cold saturated ammonium sulfate solution was added with stirring. The ammonium sulfate precipitate was collected by centrifugation at approximately 30,000 x g and resuspended in J-Buffer.

Sephadex G-100 Gel Filtration: Degassed Sephadex G-100 was equilibrated with J-Buffer, poured into a glass column and washed with the same buffer. The dialyzed ammonium sulfate fraction of the DEAE eluates was loaded onto the column and eluted with the J-Buffer. There are some technical problems associated with this step which needs standardization.

Preparative Gel Electrophoresis: The dialyzed ammonium sulfate fraction from the previous step was applied to a 5% polyacrylamide gel column and a 2.5 - 5 mA current was applied. The active fractions were collected, pooled, an equal volume of cold saturated ammonium sulfate solution was added and the tubes centrifuged at 30,000 x g. The ammonium sulfate precipitate was re-suspended in J-Buffer and stored at -20°. Portions of the purified enzyme were dialyzed before being used for further studies. This final step also needs standardization. Purity of the enzyme at each stage of the purification was checked by discontinuous polyacrylamide gel electrophoresis, although $\sim$ 120 fold purification has been achieved to date, the enzyme preparation is still quite heterogeneous.

II. Studies with Tumoral L-Asparagine Synthetase: Properties and Inhibitors of the Enzyme

Certain of the properties of the crude and partially purified tumoral synthetase have been examined during the past year. Like the enzyme from the RADA1 lymphoma, which has been studied in some detail by Meister and his colleagues, L-asparagine synthetase from the L5178Y/AR leukemia exhibits significant stimulation by chloride ions; functions as an L-glutaminase, even in the absence of ATP and L-aspartic acid; is subject to powerful product inhibition by L-asparagine, and to a lesser degree by AMP and pyrophosphate; and exhibits apparent affinities for its principal substrates comparable to the Km's reported for the RADA1 synthetase.

The systematic search for inhibitors of the L-asparagine synthetase from the L5178Y/AR leukemia, has continued during the last year. This search has been prompted by the rationale: agents which efficaciously inhibit tumoral L-asparagine synthetase(s) ought to enlarge the therapeutic scope of the oncolyic enzyme, L-asparaginase by a) preventing the emergence of resistance since the induction of L-asparagine synthetase is believed to be responsible for the resistant state; and, b) converting formerly resistant neoplasmas to sensitive variants by cancelling their mechanism of resistance.

Among the commercially-available agents recently found to produce > 50% inhibition of tumoral L-asparagine synthetase in vitro at concentrations of 1 x 10^{-3}M, or less, and not reported last year, are:

1) Thiomersal (99% inhibition)
2) NSC-163,501 - 5-Isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro (60% inhibition)
3) NSC-157,365 - Japanese Neocarzinostatin (50% inhibition)

A synthetic program for the preparation of inhibitors of L-asparagine synthetase was also begun in the past year. Because maleimide is an excellent inhibitor of this enzyme, a variety of substituted maleamic acids and monomaleimides were synthesized. Standard synthetic procedures were followed.

None of the subject compounds proved to be a significant inhibitor of the enzyme; this is perhaps due to differences in reactivity towards critical sulfhydryl functions and active N groups or to Diehls-Alder reactions. A study is underway to ascertain which mode of interaction might be most important. Included in this study is the production of ^{14}C-maleimide for use in studies of binding to, or alkylation of a partially purified preparation of L-asparagine synthetase from tumor and pancreas.

A series of dimaleimides were also prepared in order to determine whether these bifunctional agents might be more effective inhibitors of L-asparagine synthetase than their monofunctional counterparts. None, however, proved to be sufficiently superior to maleimide to warrant further study in vitro. Inhibition of pancreatic synthetase by these agents in vivo will, however, be explored.

Another potential inhibitor of the enzyme under study is L(+)-β-chloro-succinamic acid, a close steric analog of L-asparagine. The potential inhibitor was prepared according to published methods for the optical antipode. However, even at concentrations of $1 \times 10^{-2}M$, the acid was inert toward tumoral synthetase.

During this study it was thought that a maleimide coupled to an inert solid matrix might be useful for the purification of L-asparagine synthetase. Several attempts have been made to prepare a bifunctional maleimide but the drastic conditions required for the synthesis have precluded the preparation of the desired product. Alternative means will have to be tried. The last step for producing "affinity chromatographic" material will employ amino-methyl cellulose inasmuch as DEAE-cellulose can only produce quaternary functionalities which may retard enzyme binding. This hypothesis will remain unproved until both types of materials are produced. Lastly, since L-asparaginase from the serum of Dasyprocta aguti is subject to prompt and powerful inhibition by maleimide, attempts to purify it through maleimide-affinity-chromatography will also be made.

Further studies, have been undertaken in the past year to study the behavior of certain of the candidate inhibitors of L-asparagine synthetase in vivo. It has been found that mucochloric and mucobromic acids, agents which selectively and powerfully interrupt the utilization of ammonia by L-asparagine synthetase in vitro, are only feeble inhibitors of the synthetase in vivo when administered intraperitoneally at doses of up to 50 mg/kg x 1. The reason for the disparity between the in vivo and in vitro results is unknown.

Experiments also have been undertaken to examine the behavior of DON (6-diazo-5-oxo-L-norleucine), DONV (5-diazo-4-oxo-L-norvaline) and CONV (5-chloro-4-oxo-L-norvaline) on tumoral synthetase in vivo since each of these 3 agents is an excellent synthetase-inhibitor in vitro. Given by the intravenous route to tumor-bearing BDF_1 mice, only DON proved to be a significant inhibitor of tumoral L-asparagine synthetase, producing 99% inhibition after a dose of 100 mg/kg. DONV was paradoxically inert, in vivo, and caused only feeble inhibition of the biosynthesis of L-asparagine. In order to explain these discrepancies a number of hypotheses are being explored:

1) DON is not decomposed in the organism but DONV, and, to a lesser degree, CONV, are.

2) DONV and, to a lesser degree, CONV are excreted so quickly that inhibiting levels are not reached in the target tissues.

3) DON does not undergo significant protein binding, or reaction with "intercurrent" sulfhydryl functions, whereas DONV, and CONV do.

Experiments to test these hypotheses are just beginning. To date it has been found that CONV is removed from plasma with a half-life of approximately 30 seconds whereas the plasmatic half-life of DON, and DONV are approximately 4 and 5 minutes respectively. Thus clearance alone cannot explain the results given above, since DON is an excellent and DONV an inert inhibitor in vivo, while their residence-times in plasma are comparable.

In order more directly to test the hypothesis that inhibition of L-asparagine synthetase will prevent the evolution of resistance to L-asparaginase, mice bearing the L5178Y/AS leukemia have been treated concurrently with a panel of the best in vitro inhibitors of L-asparagine synthetase uncovered to date: Mucochloric acid, Ethacrynic acid, Azotomycin and Maleimide, all given daily at the 10-day maximum tolerated dose or fractions of it. A single subcurative injection of L-asparaginase was used to initiate the experiment and after an interval of one day, combination chemotherapy with the aforementioned inhibitors was begun. In animals receiving L-asparaginase alone, the tumors transitorily regress and regrow resistant to the enzyme. In animals receiving L-asparaginase and the panel of inhibitors, regression was prolonged slightly but the tumors ultimately regrew and were of the resistant variety. Analyses are at present underway to assess the extent of inhibition of L-asparagine synthetase which was, in fact, achieved with repeat-dose combination chemotherapy.

It should be pointed out that, in the L5178Y leukemia, there is more than one L-asparagine synthetase. Thus, only 60% of the enzyme present in homogenates is soluble, and a large percent of the remaining activity has been found to be associated with the nuclei. Lastly, L-asparagine synthetase is able to utilize either ammonia or L-glutamine as a source of the future amide, and most inhibitors found to date poison the utilization of only one, but not both of these precursors. For all these reasons, then, it appears to be a

difficult task to halt entirely the synthesis of L-asparagine within the tumor cell.

III. L-Asparagine Synthetase of Mammalian Pancreas

Observations made in this laboratory have established that L-asparagine synthetase is present in the pancreas of mammals at a concentration far in excess of that detectable in other organs, including liver. Studies are underway to characterize this enzyme and to compare it with its tumoral counterpart. Such investigations are of some importance from a toxicologic point of view in the light of the experiments with inhibitors of L-asparagine synthetase which were discussed above. Thus it will become important to know if normal pancreatic L-asparagine synthetase is as susceptible to inhibition as the tumoral enzyme; if it is not, some therapeutic advantage can be anticipated.

To date, studies of the pancreatic enzyme have been grouped into two main classes: physiologic and biochemical studies. Experiments were undertaken to characterize the development of pancreatic L-asparagine synthetase in the post-partum period. Neonatal pancreas has only a comparatively feeble ability to biosynthesize L-asparagine. This may reflect the abundance of L-asparagine in the casein and lactalbumin of most species. At or about puberty, there occurs a significant increase in the specific activity of the synthetase which may reflect either a change in dietary patterns and/or a hormonal effect. These pancreatic changes are the diametric opposite of those already reported to take place in the liver. Thus, antenatal hepatic tissues of the rat and chicken are rich in L-asparagine synthetase, but levels of the enzyme decline until maturity, at which time only a feeble ability to synthesize L-asparagine remains. Further studies in mice have established that an L-asparagine-free diet can augment the specific activity of L-asparagine synthetase in pancreas presumably by depletion of the product, L-asparagine, which is a powerful inhibitor of the enzyme. Additionally it has been found that agents which interrupt protein synthesis produce an accumulation of L-asparagine in the pancreas, a finding which warrants the conclusion that one important function of L-asparagine synthetase in that organ is to provide L-asparagine for protein synthesis.

Biochemical studies of the pancreatic L-asparagine synthetase in the mouse have revealed that it is similar to the tumoral enzyme catalytically. That is to say, L-asparagine is synthesized by the pyrophosphoryltic cleavage of ATP in both cases. The tumoral and pancreatic enzymes have similar mass and charge, as adjudged by electrophoresis in 3 to 12% gels of acrylamide, and are both L-glutaminases. In addition, the pancreatic synthetase shows a reaction of partial identity with the L-asparagine synthetase of the Novikoff hepatoma when exposed to an antiserum against a 45-times purified preparation of that latter enzyme in Ouchterlony plate.

At present, only a limited survey of inhibitors of the pancreatic L-asparagine synthetase has been undertaken. A few select agents which powerfully inhibited the tumoral enzyme have been examined, in vivo and in vitro versus

the pancreatic enzyme. Azotomycin and DON inhibit both to the same degree in vitro. Given in vivo, however, DON at doses of 100 mg/kg inhibits the tumoral enzyme to a greater degree than it does the pancreatic synthetase. This differential effect will be studied more systematically in the year to come.

IV. Studies with Immobilized L-Asparaginase

As was mentioned in the last annual report, L-asparaginase can be successfully coupled to Dacron Vascular Prostheses with retention of enzymatic integrity. During the past year, studies of the enzymic and immunological properties of such prostheses have been continued and largely concluded. The principal findings have been: 1) Three moles of DONV (an alkylating substrate) bind to the covalent enzyme whereas 4 moles bind to the free. This finding, if not an artifactual consequence of the process of conjugation, raises the possibility that the bound species is a trimer not a tetramer; 2) Extracorporeal perfusion of six baboons with an enzyme-prosthesis for periods of up to six hours has established that the apparatus can totally eliminate plasmatic L-asparagine. Leakage of enzyme from the devices has always been demonstrable, with a mean final concentration in the plasma of the perfused subject of 5×10^{-4} IU/ml. 3) L-Asparaginase covalently linked to Dacron is a better L-glutaminase than its free counterpart. However, whereas the free species becomes a progressively better L-asparaginase on exposure to progressively higher but sub-denaturing temperatures, the bound enzyme becomes a progressively better D-asparaginase. These findings would appear to indicate that the active site is disturbed by the process of conjugation into a non-stereospecific conformation.

V. Studies with L-Asparaginase from the Agouti, Dasyprocta aguti

Over the past three years, 24 liters of serum from the South American rodent, Dasyprocta aguti, have been collected by NCI and stored. A scheme for the partial purification of L-asparaginase from this source has been devised. Agouti serum was brought to 30% saturation with ammonium sulfate and the precipitated proteins were removed by centrifugation. The supernatant was then brought to 50% saturation by adding ammonium sulfate and the precipitated proteins, containing L-asparaginase, were collected by precipitation. The precipitate was suspended in 0.001 M Tris HCl buffer, pH 7.6, containing 0.001 M 2-mercaptoethanol, 10% glycerol and dialyzed against a large excess of the same buffer. The dialyzed ammonium sulfate fraction was loaded on a CM-Sephadex gel column and eluted with 0.001 M Tris HCl buffer, pH 7.6 containing 0.001 M 2-mercaptoethanol and 10% glycerol. Preparative gel electrophoresis of the CM-Sephadex gel eluate is being investigated. There are salient problems with the stability of the enzyme which are also being examined. Various other steps such as acetone or alcohol precipitation, DEAE and CM-cellulose ion exchange chromatography, DEAE-Sephadex and Sephadex gel columns also were explored. Affinity chromatography using agarose to which L-aspartic acid and L-asparagine are linked via an alkyl chain were investigated but these materials did not yield reproducible purification.

During the course of this study the problem of measuring L-asparaginase in numerous samples was solved by devising a facile method of assay which was sensitive and convenient.

VI. Studies with Azotomycin

During the past year, studies with Azotomycin, a diazoketone antibiotic with good antitumor activity versus L1210 et al., but with prohibitive gastrointestinal toxicity in man, have been concluded. It was found that the agent behaves as a 'pro-drug', liberating DON in vivo as a consequence of peptidasic attack. DON then interrupts the utilization of L-glutamine in the numerous (12-14) known reactions entailing amide transfer from that amino acid. L-Asparagine synthetase will be recalled as one such reaction.

An interesting pathologic change produced by Azotomycin has been studied in some detail: mice given large doses of the drug show excitability grading into ataxia followed by epileptiform convulsions and concluding with preterminal depression. A histopathologic basis for these changes was sought. Marked vacuolar degeneration of the nucleus fastigii at the base of the cerebellum was found to be present in intoxicated mice but not in controls. Electrocorticograms of the subjects given 900 mg/kg of Azotomycin were taken therefore and the encephalographic changes were found to be wholly consonant with the clinical picture - that is to say, starting 4 hours after dosing - with isolated spikes, the electrocorticogram developed into a typical epileptic pattern which was supplanted by a low-voltage profile as soon as 31 hours.

When a biochemical basis for this phenomenon was sought, it was found that L-glutamate, a putative neurotransmitter, had accumulated in the brains of treated subjects, and that L-asparagine synthetase was rendered largely inactive as a consequence of treatment with the drug.

VII. Studies with Streptozotocin

In the last annual report, it was documented that the diabetogenic oncolytic antibiotic Streptozotocin (NSC-85998) given intravenously to mice at doses of 1000 and 500 mg/kg, brought about alkylation of DNA from liver and kidney at its guanyl residues. In the present year this finding has been confirmed. In addition, studies are underway to determine whether nicotinamide - the vitamin which counteracts the diabetogenicity of Streptozotocin - will prevent the alkylation. Lastly, isolated islets of Langerhans are being accumulated in large numbers from animals given diabetic doses of Streptozotocin in order that the degree of alkylation, if any, of this important target-tissue can be assessed.

Significance to Bio-medical Research and the Program of the Institute

L-Asparaginase continues to be an important and effective form of chemotherapy for select lymphatic neoplasms. If powerful inhibitors of L-asparagine

synthesis can be uncovered, the therapeutic scope of L-asparaginase ought to be broadened to include other tumors whose mechanism of resistance to the enzyme entails a significant autochthonous ability to synthesize L-asparagine. The use of such inhibitors can be anticipated to also produce substantial toxicity to the host unless some fortuitous tumor specificity resides in the molecule. For this reason, comparative studies of the influence of the candidate inhibitors on normal pancreatic L-asparagine synthetase, as contrasted with the tumoral enzyme, are of considerable importance from a toxicologic point of view. Lastly, because the role which the L-glutaminasic activity of L-asparaginase from E. coli plays in its therapeutic action has never been clearly ascertained, the purification of the L-asparaginase from agouti serum is being attempted. It is hoped that this L-glutaminase-free enzyme can be used as a reference tool for answering certain fundamental questions on oncolysis with enzymes.

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Serial No. NCI-6110
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1972 to June 30, 1973

Project Title: The Pathology of Drug-Induced Alterations in Animals and Man

Previous Serial Number: Same

Principal Investigator: David M. Young

Other Investigators: Harry M. Olson
David J. Prieur
Louis M. Buja
Roger S. Jaenke

Cooperating Units: Laboratory of Pathology, DCBD, NCI
Section of Pathology, OD, NHLI
Colorado State University

Man Years:
Total: 2 1/2
Professional: 1 1/2
Other: 1

Objectives:

The objectives of this project are:
1) To conduct scientific investigations designed to elucidate the mechanism(s) of antineoplastic drug-induced pathologic alterations.

2) To provide guidelines to standardize, semi-quantitate, and computerize pathologic evaluation of experimental animals utilized by contractors in preclinical toxicologic testing of antineoplastic agents.

3) To systematically evaluate and record reports of adverse effects of antineoplastic agents in man on a computerized basis and correlate these findings with those recorded in test animals.

Principal Findings:

The Pathogenesis of L-Asparaginase-Induced Hypocalcemic Tetany in Rabbits

Additional studies of L-asparaginase (asnase)-induced tetany in rabbits have contributed to a further understanding of the manifestations of the drug induced disorder. The role of asnase-induced azotemia in the development of hypocalcemia in the rabbit was investigated. Performance of bilateral ureterectomies resulted in elevations of BUN values up to 16 times normal

without either incidence of significant hypocalcemia or tetany. Since asnase derived from E. coli contains an endotoxin contaminant, studies were conducted to determine the role of endotoxin in the hypocalcemic disorder. Single doses of endotoxin administered at levels 100 fold greater than that present in a single dose of asnase utilized in the study produced only a mild hypocalcemia without any evidence of tetany. These results indicate that neither endotoxin nor the uremic effects of asnase are etiologic in the development of the experimentally induced hypocalcemic tetany. The aforementioned findings in combination with histopathologic and ultrastructural evidence of degenerating chief calls, degranulation, autophagy and deficiency of subcellular organelles associated with protein synthesis in parathyroid glands of treated rabbits offer support for the hypothesis that the parathyroid lesion is related to the cause, rather than an effect, of the hypocalcemia. The rabbit-asnase system is presented as an experimental animal model of drug-induced hypoparathyroidism characterized by drug-endocrine cell interactions somewhat analogous to the lethal selectivity of alloxan for pancreatic beta cells in production of experimental diabetes.

Daunomycin/Adriamycin-Induced Cardiomyopathy

Considerable intramural and extramural investigative efforts have been conducted with the basic objective to define an animal model which develops clinicopathologic manifestations of cardiotoxicity similar to that reported in treated cancer patients. Recent findings indicate that the rabbit is an ideal model for anthracycline-induced cardiomyopathy. Rabbits treated with adriamycin develop a characteristic syndrome of congestive heart failure with subcutaneous edema, hydrothorax, ascites, hydropericardium, and morphologic alterations in the myocardium. Currently biochemical and electron cytochemical techniques are being developed to further define the pathogenesis of the anthracycline-induced cardiomyopathy in the rabbit.

A systematic analysis of myocardial tissue from cancer patients treated with daunomycin was conducted recently by Dr. Buja and colleagues. They characterized the myocardial lesion electron microscopically and correlated these findings with in vitro studies of daunomycin treatment of monkey heart cultures. Ultrastructural alterations in myocardial cells in both in vivo and in vitro studies had many characteristic similarities. These workers emphasize the significance of ultrastructural nuclear changes whereas mitochondrial alterations appear to be of major consideration in the rabbit model. Collaborative experiments are in progress to further define the ultrastructural similarities and dissimilarities of the anthracycline-induced myocardial lesion in man and the rabbit.

Pathologic and Therapeutic Effects of Cancer Treatment

Preliminary findings from a retrospective study conducted during 1971 of cancer patients treated at the NIH Clinical Center revealed that it would be advantageous to systematically evaluate the pathologic and therapeutic effects of cancer treatment. A number of programs designed to process and

computerize pathology data were investigated. The major limitations in employment of existing programs concerned utilization of either numerical analysis through manual coding of medical terms or "key-word" substitution analysis. These limitations were circumvented by use of the Systematized Nomenclature of Pathology (SNOP) as developed by the College of American Pathologists and supported by NIH. The SNOP system allows the investigator to enter information in natural language and the computer encodes the medical data into four basic fields. A special computer program was developed in cooperation with the Laboratory of Pathology, DCBD, NCI and DCRT, NIH, to assimilate and store pathologic and therapeutic data in a retrievable format on cancer patients. The program tabulates and codes data concerning patient identification, clinical diagnoses, pathological diagnoses, therapeutic modalities (surgery, radiation, chemotherapy, immunotherapy, supportive care) and ancillary information (i.e. dose, route of administration, schedule, duration of treatment, adverse reactions), therapeutic chronology and terminal data (i.e. nutritional status, remission status, neoplasia evident at death, undiagnosed neoplasia present, septicemia, cause of death). Autopsy reports on cancer patients are reviewed, summarized and entered in the computer on a weekly basis.

The aforementioned computerized program has been in use for the past six months and currently data on a total of 190 patientsis evaluable. Preliminary analysis of the material in the data base revealed that clinical and pathological (autopsy) diagnoses were in agreement in over 90% of the cases. These findings are in contrast to those reported recently by Bauer and Robbins (JAMA 221: 1471, 1972) of incorrect diagnoses occurring in 41% of all cancers in a large autopsy study series. Of the cancer patients reviewed at NIH in the current study, 22% had neoplastic processes evident at autopsy other than originally diagnosed. In addition preliminary findings indicate that the occurrence of additional primary neoplasms may not be uncommon in patients receiving antineoplastic therapy for breast cancer. These results correlate well with the recent report by Davis et al. (Cancer 31: 543, 1973) of acute leukemia complicating metastatic breast cancer in patients maintained on prolonged chemotherapy.

Considering the rise in popularity of the use of aggressive modalities of cancer chemotherapy and long term administration for maintenance, it is of interest to establish a thorough understanding of the pathologic and therapeutic effects of treatment. The present program, currently in early stages of development, is designed to correlate antemortem occurrences with postmortem findings. Questions of concern are: a) What is the most common cause of death in cancer patients receiving therapy? b) Is there a correlation between various modalities of therapy and specific pathologic findings at autopsy that would assist in alerting the clinician of impending adverse effects and complications in future cases? and c) What is the occurrence of false positives and false negatives in predicting for adverse effects in man from data obtained in preclinicial toxicologic studies in dogs and monkeys?

Standardization of Pathologic Evaluation of Dogs and Monkeys Utilized for Preclinical Toxicologic Testing of Anticancer Agents.

During 1972 the preclinical toxicologic protocols of the Laboratory of Toxicology were reviewed extensively and appropriate revisions were incorporated. A significant alteration in the pathology protocol involved conversion of the previously recommended lexicon to SNOP, a more universal standard. Each contractor's pathologist reports pathologic findings by coding specific diagnoses according to the SNOP lexicon. The keysort cards; one for each animal on test, are submitted to the Laboratory of Toxicology in conjunction with the final preclinical toxicology report on each drug. The procedure enhances computerization and storage of data at NIH. In addition, utilization of similar computerized systems for both human and animal data in toxicologic studies expedites retrieval of information on a comparative basis.

Significance to Bio-Medical Research and the Program of the Institute:

The need for a better understanding of the pathologic effects of anticancer agents cannot be overemphasized. Upon elucidation of the pathogenesis of drug toxicity, methods can be explored to nullify the adverse effects while retaining the drug's anticancer activity. A by-product of these studies of drug-induced lesions is the possible definition of new experimental models to study spontaneous disease in animals and man. For example, Daunomycin/Adriamycin-induced cardiotoxicity may prove to be an excellent model to study the pathogenesis of congestive heart failure and asnase-induced hypocalcemic tetany in the rabbit may provide the first experimental model of selective parathyroid cell destruction by a chemical agent to study the mechanism of calcium homeostasis.

Comparison of drug-induced lesions in man and animals will assist in the critical evaluation of predictability for specific adverse effects utilizing a carefully designed preclinical toxicologic protocol. In addition, standardization of reporting pathologic effects of anticancer drugs will expedite transfer of agents through the Laboratory of Toxicology in the Linear Array of the Chemotherapy Program.

Proposed Course:

The present investigations will be continued and expanded to better assess the pathologic effects of anticancer agents. The computerization and storage of data derived from evaluation of pathologic and therapeutic effects of cancer treatment in man and pathologic evaluation of dogs and monkeys utilized for preclinical toxicologic studies will be handled by a prime contractor in the near future. These computerized programs both use the SNOP nomenclature and have been developed to a stage at which it would be advantageous to have the day to day tabulation and storage performed on an extramural basis. The Laboratory of Toxicology plans to assess the performance of pathologic evaluation by the contractors through use of a quality

control system to be developed in the next year.

Intramural research activity will be directed towards defining mechanisms of delayed toxicity in the anthracycline-rabbit model. Specific studies are being designed currently to determine the role of altered mineral metabolism in the development of the myocardial lesion in the rabbit. Additional studies will be performed to characterize a recent finding of retardation of osteogenesis in rabbits on long term adriamycin therapy.

Studies are being designed currently to define the distribution of L-asparaginase in different organs of the rabbit. These investigations may contribute to a better understanding of L-asparaginase-induced hypocalcemia by determining whether distribution of the radioactive drug correlates with ultrastructural alterations in the parathyroid glands.

Publications:

1. Rowland, G. N., Capen, C. C., Young, D. M. and Black, H. E.: Microradiographic Evaluation of Bone from Cows with Experimental Hypervitaminosis D, Diet-Induced Hypocalcemia, and Naturally Occurring Parturient Paresis. Calc. Tissues Res. 9: 179-193 (1972).

2. Prieur, D. J., Young, D. M., Davis, R. D., Cooney, D. A., Homan, E. R., Dixon, R. L., and Guarino, A. M.: Procedures for Preclinical Toxicologic Evaluation of Cancer Chemotherapeutic Agents: Protocols of the Laboratory of Toxicology. Cancer Chemother. Rep. In Press (1973).

3. Black, H. E., Capen, C. C., and Young, D. M.: Ultimobranchial Thyroid Neoplasms in Bulls. A Syndrome Resembling Medullary Thyroid Carcinoma in Man. Cancer. In Press (1973).

Serial No. NCI-6113
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1972 through June 30, 1973

Project Title: The Detection, Quantitation, Evaluation, and Prediction of the Pathologic Effects, Especially on the Hematic System, of the Toxicity of Antineoplastic Agents and Studies of Other Conditions of Host-Defense Failure

Previous Serial Number: Same

Principal Investigator: David J. Prieur

Other Investigators: David M. Young
Harry M. Olson

Cooperating Units: Division of Cancer Treatment

Man Years:

Total:	2
Professional:	0.5
Others:	1.5

Objectives:

1. To detect, quantitate and evaluate the pathologic effects of anticancer agents.

2. To elucidate the mechanisms of these toxic effects.

3. Relate the toxic effects on the hematic system to the host-defense failure in patients treated with the agents.

Project Description:

During the past year studies were initiated and performed to detect, quantitate, evaluate, and predict the effects of antineoplastic agents on the hematic system. In addition to the aforementioned studies, a major effort was expended in clarifying, updating, and revising the protocols of the Laboratory of Toxicology that are used by the contractors of the laboratory in performing the preclinical toxicologic studies on antineoplastic agents.

Major Findings:

The treatment of animals with antineoplastic agents that adversely affect bone marrow and leukocytes did not lead to the anticipated elevation of

serum levels of the leukocytic enzyme muramidase. Subsequent studies showed however that compared to man the levels of the enzyme muramidase are relatively low in the serum and leukocytes of rabbits and dogs. The usefulness of the Pseudomonas polysaccharide Piromen in the measurement of the bone marrow reserves of dogs has been verified.

Significance to Biomedical Research and the Program of the Institute

Because most of the antineoplastic agents have detrimental effects upon the hematic tissue and especially the leukocytic tissue of treated patients, these persons are rendered quite susceptible to infectious disease. Some of the persons cured of neoplastic disease by these agents succumb to infectious disease because of their increased susceptibility. In the current preclinical toxicology, the primary methods of estimating bone marrow toxicity are the total and differential white blood cell counts. There are several reasons however why these techniques are inadequate to accurately quantitate the toxicity of an antineoplastic agent in preclinical toxicologic studies and to predict the toxicity of the agent in man. These reasons are related to the fact of the large size of the bone marrow reserves of mature leukocytes and the presence of a marginated leukocytic pool in the blood vessels of the body. Muramidase is an enzyme which occurs in high levels in neutrophils and monocytes; the primary phagocytic cells of the blood. If these cells are killed in greater than normal numbers as would be the case in animals treated with many of the antineoplastic agents, the levels of the enzyme in the serum should rise. This type of response has been observed in man. In dogs, the primary animal used in preclinical toxicology, there was, however, no clear-cut rise in serum muramidase. Subsequent studies have demonstrated that at least part of the reason for this type of response is the relatively low level of muramidase in the leukocytes of dogs compared to man. Piromen is one of a group of endotoxin-like agents which can be used to measure the bone marrow granulocyte pool. This drug causes the release of the granulocytes into the circulation where they can be obtained and measured. Applied judiciously to dogs treated with antineoplastic agents this technique should allow the identification and more precise quantification of subacute toxicity to bone marrow. A problem with this technique that will probably prevent its routine application is that exercise and excitement also cause a rise in circulating granulocytes and these two conditions are almost always associated with the handling of laboratory dogs. It is possible that by proper conditioning this problem can be overcome.

The revision of the protocols of the Laboratory of Toxicology have been completed. These revisions, clarifications, and corrections will undoubtedly lead to better preclinical toxicologic data from the contractors of the Laboratory of Toxicology. There will be more standardization of both actual laboratory procedures and presentation of data in reports between the various contractors of the Laboratory. This will allow the staff of the Laboratory to more accurately predict the clinical toxicity of an agent. The revised protocols are also essential in the acquisition of a prime contractor for the Laboratory of Toxicology.

Future Work

The areas of laboratory work enumerated above will be continued and expanded during the coming year. Studies on the enzyme muramidase will be performed in rabbits and dogs and studies concerning the measurement of bone marrow reserves will be continued in dogs.

Publications:

1. Prieur, D. J., Young, D. M., Davis, R. D., Cooney, D. A., Homan, E. R., Dixon, R. L., and Guarino, A. M.: Procedures for Preclinical Toxicology Evaluation of Cancer Chemotherapeutic Agents: Protocols of the Laboratory of Toxicology. Cancer Chemotherapy Rep., 1973 (IN PRESS)

Serial No. NCI-6122 (New)
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1972 through June 30, 1973

Project Title: Drug interactions and other factors influencing the physiological disposition and toxicity of drugs.

Principal Investigators: C. L. Litterst, T. E. Gram, A. M. Guarino

Other Investigators: J. R. Althaus, R. L. Reagan and E. R. Homan

Cooperating Units: None

Man Years:

Total:	4.2
Professional:	3.2
Other:	1

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon an intricate balance between organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins and the rate of excretion into the urine and bile. Moreover, the metabolic clearance of drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, when working in a dynamic equilibrium, absorption, distribution, binding, metabolism, and excretion tend to dictate the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess quantitatively these parameters is of obvious therapeutic significance. These are the goals against which the Drug Interaction Unit was organized. Although this unit has been in existence for less than a year, a number of studies are underway which offer promise of both scientific import and relevance to the NCI.

Methods Employed:

Standard enzymatic, analytical, and surgical techniques have been utilized.

Major Findings:

A. The distribution of CCNU in rabbit tissues following intravenous injection. The nitrosoureas are poorly soluble in aqueous media and as a result dose forms for parenteral administration have relied upon alcohols, such as ethanol or propylene glycol and upon emulsions. Since the physical form in which a drug is deposited in the vascular space influences its pharmacokinetics, the distribution of ^{14}C-CCNU in rabbit tissues was investigated employing three vehicles: propylene glycol:ethanol (4:1), intralipid:saline (1:9), emulphor:ethanol:saline (1:1:19). Fifteen tissues and three body fluids were assayed for radioactivity at 10 minutes, 1, 4, 12 and 24 hours after administration of 1 mg/μCi/kg of ^{14}C-CCNU. By 12 hours after administration, the drug had been eliminated from all tissues except kidney and liver, but was still detectable in bile and urine. Four hours after administration the tissues containing the largest amounts of radioactivity were lung, kidney, and liver. Drug-derived radioactivity was present in adrenal glands in very high levels 10 minutes after administration. Large amounts of radioactivity were excreted into the bile. There were no large differences in distribution except for increases in levels of activity in body fat when emulphor and intralipid were used. The major effect of different vehicles centers around how rapidly initial levels of drug decay from a particular tissue.

B. The influence of selected antineoplastic agents on drug metabolism by enzymes in hepatic microsomes. Single doses of a number of antineoplastic drugs produce a roughly dose-dependent inhibition of microsomal drug metabolism in rats and mice. With the exception of the nitrosoureas whose effects persist for at least five weeks, the enzyme inhibition is most profound during the first 3-4 days and enzyme activities generally return to control levels in 1 to 2 weeks. Studies with other drugs such as phenobarbital have shown that daily administration for 3 to 7 days produces a profound stimulation of microsomal enzyme activity with a concomitant reduction in the duration or intensity of action of the drugs. It is of interest that many such enzyme "inducers" exhibit biphasic effects on microsomal enzymes, i.e., an initial impairment or inhibition followed later by stimulation. Although single doses of many carcinolytic agents had been shown to inhibit microsomal enzymes, the possibility that multiple doses might be associated with enzyme induction has not been explored adequately. Our plan was therefore to administer daily therapeutic doses of several antineoplastic agents for 5 days and to examine microsomal enzyme activities at several time points afterward (1, 5, 10, 14 days). Male CDF_1 mice were treated with the following drugs: chlorambucil, 5-fluorouracil, Me-CCNU, BCNU, adriamycin or daunomycin. Preliminary experiments have not yielded consistent results due in part to problems of delayed toxicity and the very steep dose-response curves for these agents. However, this problem has been resolved by a recent intensive dose-response and survival duration determination.

C. Effect of microsomal enzyme induction on the acute toxicity of drugs. Pretreatment of animals for 3-5 days with phenobarbital (Pb) markedly shortens the duration and intensity of action of many other drugs. These effects

generally involve decreases in the duration of drug action, by the use of "pharmacological" doses. However, the question of whether Pb pretreatment alters the effects of larger toxic doses, i.e. the LD_{50}, has been largely ignored. Male CDF_1 mice were injected with either saline or phenobarbital (75 mg/kg) once daily for 4 days. Twenty-four hours after the last dose, animals were injected intraperitoneally with a range of doses of either p-nitrophenol, ethylmorphine, aniline, or antipyrine and the 24 hour LD_{50} determined. Similarly treated animals were sacrificed and the activities of the following hepatic microsomal enzymes determined: aniline hydroxylase, ethylmorphine demethylase and cytochrome P-450. Preliminary results indicated that the Pb pretreatment produced the expected increases (50 to 300%) in enzyme activities. However, there was a dramatic increase in the LD_{50} especially for ethymorphine.

D. Hepatic microsomal drug metabolism in bats. Species differences in drug metabolism are an important determinant in inter-species variations in sensitivity and toxicity to xenobiotics. In light of this information hepatic drug-metabolizing enzyme activities in two species of eastern American bats [Myotis lucifugus and Plecotus rafinesquil] were examined and found to be qualitatively similar to most other mammals. Bat liver microsomes had slightly lower levels of cytochrome P-450, but up to 80% less ethylmorphine demethylase and NADPH cytochrome with reductase activity. Ultrastructurally, liver cells of bats showed abundant mitochondria, many of which showed distinct configurational differences. Endoplasmic reticulum in bat liver cells was very sparse relative to most mammals. Electron micrographs of microsomal suspensions from bats demonstrated a relative lack of smooth membranes and the microsomes from Myotis lucifugus exhibited an uncommon non-vesiculated appearance.

E. Acute toxicity of Δ^9-tetrahydrocannabinol administered intravenously. As a corollary of the distribution study in which ^{14}C-CCNU was administered intravenously to rabbits in three different vehicles, experiments were conducted to study the toxicity of Δ^9-tetrahydrocannabinol (THC) when administered intravenously to rabbits. The vehicle for the THC administration was emulphor:ethyl alcohol:saline(1:1:19, v/v). The administration of 100 mg/kg of THC in 10 ml of vehicle produced intense peripheral vasodilatation, very rapid, shallow breathing and extremely hyperactive behavior with a characteristic stiff-legged, tip-toe gait. Several animals died at this dose. THC at 40 mg/kg in 10 ml of vehicle produced hyperactive behavior but no lethability.

F. Biliary excretion of glucuronides in Wistar Gunn rats. Male rats of the Wistar strain and male homozygotes of the Gunn mutant strain, deficient in UDP-glucuronyl transferase, were prepared surgically and examined for free and conjugated phenol red(PR) excretion into bile. The percentage of the administered dose recovered as free PR in the bile was identical for the two strains. While one third of the PR administered to Wistar rats was recovered as a biliary conjugate no significant levels of the glucuronide appeared in bile from Gunn rats. Identification of conjugated PR in the bile of Wistar rats as the glucuronide was confirmed

by cleavage with β-glucuronidase and thin layer chromotography in two solvent systems. Gunn rats concentrate free PR in the bile less effectively than the parent strain. One important observation is the similarity of the deficiency in phenol red excretion patterns in hmozygous and heterozygous Gunn rats. Preliminary experiments have been done with hycanthone, ellipticine and 9-methoxy-ellipticine in an effort to apply this technical approach to studies of biliary excretion of oncolytic drugs and their metabolites.

G. Renal and hepatic excretion of xenobiotics. Because of the proposed congruence of renal and hepatic excretory functions in mammals, we have been studying similar functions in the dogfish shark, Squalus acanthias. This marine species was chosen because the transport and metabolic processes occur at slower rates than in mammals. We feel this is particularly important in studies of the rather labile antineoplastic drugs. Therefore, because of this opportunity to study both transport and metabolism "in slow motion"; 25 compounds were studied in this manner and included the following antineoplastic agents: CCNU (ring labeled), CCNU (side chain labeled), BCNU, daunomycin, adriamycin, actinomycin D, puromycin and MTX. In general it was found that the lipid soluble, low molecular weight oncolytic agents, such as BCNU and CCNU were not excreted in urine or bile, but presumably were excreted via the gills. Higher molecular weight agents such as daunomycin, adriamycin, MTX, puromycin and actinomycin D were excreted both in urine and bile with the latter usually dominating.

Significance to Biomedical Research and the Program of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of a drug will become either a subthreshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in an era of combination drug administration, cannot be overemphasized.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we plan to study the metabolic fate of antineoplastic agents. It is anticipated that we can make use of carcinolytic agents as model substrates for in vitro measurements of enzyme activity. The biliary excreting model will be extended to study excretion of antineoplastic agents and their metabolites with subsequent identifcation by mass spectral techniques.

Publications:

1. Guarino, A.M. and Litterst, C.L.: Metabolism of cancer chemotherapeutic agents via pathways utilized by xenobiotics. In: Handbook of Experimental Pharmacology - Antineoplastic and Immunosuppressive Agents. A. Sartorelli and D.G. Johns (Eds), Springer-Verlag Co., Heidelberg, 1973 (IN PRESS).

2. Cradock, J.C., Davigon, J.P., Litterst, C.L. and Guarino, A.M.: An intravenous formulation of Δ^9-tetrahydrocannabinol using a nonionic surfactant. J. Pharm. Pharmacol. 25, 1973 (IN PRESS).

3. Guarino, A.M., Anderson, J.B., Starkweather, D.K. and Chignell, C.F.: Pharmacologic studies of sodium camptothecin (NSC-100,880): Distribution, plasma protein binding, and biliary excretion. Canc. Chemother. Rep. 57, 1973 (IN PRESS).

4. Litterst, C.L., Reagan, R.L., and Gram, T.E.: Microsomal drug-metabolizing enzyme activity in livers of bats. Comparative Biochemistry and Physiology, 1973 (IN PRESS).

5. Hook, G.E.R., Bend, J.R., Hoel, D., Fouts, J.R., and Gram, T.E.: Preparation of lung microsomes and a comparison of the distribution of enzymes in subcellular fractions of rabbit lung and liver. J. Pharmacol. Exp. Therap. 182: 474-490, 1972.

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7. Chabra, R.S., Gram, T.E., and Fouts, J.R.: A comparative study of two procedures used in the determination of hepatic microsomal aniline hydroxylase. Toxicol. and Appl. Pharmacol. 22: 50-58, 1972.

8. Gram, T.E.: Separation of hepatic smooth and rough microsomes associated with drug-metabolizing enzymes. In Methods in Enzymology. R.W. Estabrook, L. Packer and S. Fleischer (Eds), 1973 (IN PRESS).

9. Gram, T.E.: Comparative aspects of mixed function oxidation by lung and liver of rabbits. Drug Metabolism Reviews, 1973 (IN PRESS).

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11. Holtzman, J.L., Erickson, R.R., Gram, T.E., Dewan, R.K., and Bloomfield, V.A.: Kinetics and inelastic light scattering studies of the role of phospholipids in the microsomal membrane structure. Drug Metabolism and Disposition 1, 1973 (IN PRESS).

12. Litterst, C.L., Farber, T.M., and VanLoon, E.J.: Potentiation of CCl_4 induced hepatotoxicity in one drug by chronic exposure to phenobarbital. Tox. Appl. Pharm. 1973 (IN PRESS).

13. Litterst, C.L. and VanLoon, E.J.: Enzyme induction by PCB relative to known inducing agents. Proc. Soc. Expt. Biol. Med. 141: 764-768, 1973.

14. Pritchard, J.B., Guarino, A.M., and Kintz, W.B.: Distribution, metabolism and excretion of DDT and mirex by a marine teleost, the cointer flounder. Env. Health Perspective, 3, 1973 (IN PRESS).

15. Goldin, A., Carter, S., Homan, E.R., and Schein, P.S.: Quantitative comparison of toxicity in animals and man. The design of clinical trials in cancer chemotherapy. (Ed. M. Staquet) Editions Scientifiques Europeenes, Brussels, 1972, pp. 58-81.

16. Goldberg, A., Cooney, D.A., Glynn, J.P., Homan, E.R., Gaston, M.R., Milman, H.A.: Effects of immunization to L-Asparaginase on antitumor and enzymatic activity. Cancer Res. 33:256-261, 1973.

17. Prieur, D.J., Young, D.M., Davis, R.D., Cooney, D.A., Homan, E.R., Dixon, R.L., and Guarino, A.M.: Procedures for preclinical toxicologic evaluation of cancer chemotherapeutic agents: Protocols of the Laboratory of Toxicology. Cancer Chemother. Repts., 4(3):1-29., 1973.

18. Cooney, D.A., Homan, E.R., Cameron, T., Schaeppi, U.: Measurement of 4-(14-C)-L-asparagine in body fluids and tissue: Methodology and application. J. Lab. Clin. Med. 1973 (IN PRESS).

19. Homan, E.R. and Dixon, R.L. Prediction from laboratory animals to man - quantitative relationships. Proc. Fifth Intern. Congr. on Pharmacol., San Francisco, California, 1972. 1973, (IN PRESS).

20. Neil, G.L., Kuentzel, S.L., Moxley, T.E., and Homan, E.R.: The effect of dose interval on the survival of L1210 leukemic mice treated with DNA synthesis inhibitors. Cancer Research 1973, (IN PRESS).

Serial No. NCI-6123 (New)
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1972 through June 30, 1973

Project Title: Computer Methods for Quantification of Structure - Activity Relationships

Previous Serial Number: None

Principal Investigators: A. M. Guarino, K. H. Ting and K. C. Chu

Other Investigators: H. Fales, T. E. Gram, R. C. T. Lee, G. W. A. Milne, M. B. Sharpiro and J. M. Venditti

Cooperating Units: Drug Research and Development, Division of Cancer Treatment, Division of Computer Research and Technology, Laboratory of Chemistry, and National Heart and Lung Institute

Man Years:

Total:	3.5
Professional:	3.5
Others:	None

Project Description:

Ever since Meyer and Overton observed a correlation between lipid solubility and anesthetic potency of a series of drugs, many other physical parameters have been studied in an effort to discern other possible relationships between physical properties and a given pharmacologic effect. During the past year two projects, much more sophisticated in nature, have been initiated.

The objective of the first project is to examine the relationships between the mass spectrum and pharmacologic activity of a drug molecule. The purpose of the second project is to develop a computer-assisted method for predicting how a new drug may be metabolized by the body.

Principal Findings:

Relationships between mass spectra and pharmacologic activity of drugs

This possible relationship was investigated with the help of various computer-based pattern-recognition techniques. By means of these techniques a datum point can be located on grid coordinates and when many of these points are related in some way, they will cluster. In our preliminary experiments, designed to develop the necessary models, we studied the relationships between the mass

spectra and the biologic activities of two closely related drug classes, sedatives and tranquilizers. In testing 66 drugs, using each in turn as the unknown and the remaining 65 as knowns, it was found that at least 80% of the drugs clustered into two primary groups and therefore were classified correctly. Further refinements of the computer methodology lead to even greater accuracy of predictions with minimal residual overlap between drug classifications. When the mass spectra of drugs not classified as sedatives or tranquilizers, (e.g. hallucinogens) were incorporated into the program, they invariably clustered in an intermediate area between the two primary classes. In an effort to further challenge this system, we entered the mass spectrum of diazepine (NSC-66020), a benzodiazepine analog with poor tranquilizing activity but good antineoplastic activity in the L1210 system. Very interestingly, this drug did not cluster in the major area of the tranquilizers. Although this present method could only predict the negative point, i.e. that the agent was not a tranquilizer, we are building a data base of compounds with antineoplastic activity so that shortly we will be able to classify for this category of pharmacologic activity.

Predicting metabolism of drugs by computer assisted methods

This project was initiated toward the end of the present fiscal year. In the early phase of this work, the Corey Program for computer recognition of structural features of organic chemicals, is being revised to handle the many unique structures found in drug molecules. By using existing knowledge, both from the literature and from the intuition of people established in the field, a hierarchy is being developed for primary and secondary reactions which occur in drug metabolizing pathways. The present emphasis is on one species, the rat, and considers overall drug metabolism as measured by the nature of the biotransformation products which reportedly are excreted.

Significance to Biomedical Research and the Program of the Institute

Although the Drug Development Program makes extensive use of computers in the acquisition, storage and retrieval of information on the antineoplastic drugs, there has been little effort in the direction of using computers as learning and predictive devices. The present studies are based on the hypothesis that since the ultimate mechanism by which a drug exerts its pharmacologic or toxicologic action is by interaction of the drug molecules with macromolecular receptors in the body, then the mass spectral fragments pattern may indicate more precisely which parts of the drug molecule are necessary for the biologic action. Therefore, it is thought that once the mass spectral characteristics which are associated with good anticancer activity are established, the following can be done: 1) predict drug activity from the structure (mass spectrum); 2) estimate which compounds from a series of analogs would have the greatest oncolytic activity; 3) suggest to the chemist which structural features should be built into a drug molecule to obtain maximum antineoplastic activity, and 4) substitute "toxicity" data for pharmacologic "activity" and thus develop knowledge for understanding which structural moieties are toxic and therefore to predict and synthesize drugs designed to minimize toxicity and enhance therapy.

The computer prediction of metabolic pathways for drugs is particularly significant for antineoplastic agents because, compared with other classes of drugs, this area is in relative infancy. Such studies for other classes of agents are part of preclinical testing, but the innate chemical instability and lack of analytic methodology have hampered work on the oncolytic drugs. Hence the development of a computer based system would aid such studies by suggesting which of dozens of possible metabolites should be sought first.

Proposed Course

The mass spectra of many nitrosoureas will be classified according to their relative potency in the L1210 screen. Correlations will be sought between the mass spectrum and biological activity with the aim of selecting those molecular features most prominantly associated with oncolytic effects. Although four such agents are already in the clinic, we may discover that some of the other drugs may have properties suggesting it should be tested for possible greater clinical activity than those already in the clinic. Considerably more work will be done to broaden the data base on the metabolism of drugs, emphasizing primarily what is known about the oncolytic agents.

Publication:

1. Ting, K.H., Lee, R.C.T., Milne, G.W.A., Sharpiro, M.B. and Guarino, A.M.: Applications of Artificial Intelligence. Relationship between mass spectra and pharmacological activity of drugs. Science 180, 1973 (IN PRESS).

SUMMARY OF LABORATORY OF TOXICOLOGY
SUPPORTED CONTRACT ACTIVITIES
July 1972 - June 1973

Completed Protocol Type Studies			INDA Submitted
1.	NSC-15200	Gallium Nitrate	May 1973
2.	NSC-68075	Thalicarpine	August 1972
3.	NSC-71851	-TGdR	May 1973
4.	NSC-77037	Tetrandrine (+)	March 1973
5.	NSC-68075	L-Alanine, 3-(tritylthio)-	No Phase I Protocol received
6.	NSC-95441	Me CCNU - intralipid vehicle	Amendment filed May 1972
7.	NSC-121,210E	DHEA Mustard	No Phase I Protocol received
8.	NSC 135,758	Piperazinedione crystalline antibiotic	No Phase I Protocol received
9.	NSC-1136,20E	Androst-4-en-3-one, 17β-hydroxy-7β-methyl-, propionate	No Phase I Protocol received
10.	NSC-139,105	Baker's antifol	May 1973
11.	NSC-143,769	MER BCG	April 1973

SPECIAL STUDIES COMPLETED

Completed Protocol Type Studies			
1.	NSC-67574	Vincristine	IV Retrospective Study employing clinical route and regimen
2.	NSC-69856	Neocarzinostatin	Comparison with Japanese Neocarzinostatin
3.	NSC-77213	Procarbazine	PO Reptrspective Study employing clinical route and regimen
4.	NSC-82151	Daunomycin	IV Reptrospective Study employing clinical route and regimen
5.	NSC-102,816	5-Azacytidine	Hamster cheek pouch
6.	NSC-112,907	Tetrahydrouridine	Single dose study in dogs and monkeys
7.	NSC-119,875	Platinum cpd.	Ototoxicity in guinea pigs and monkeys
8.	NSC-119,875	Platinum cpd.	Renal toxicity in mice
9.	NSC-125,066	Bleomycin	Zinc levels in body fluids and tissues
10.		Emulphor	Vehicle Toxicity

ONGOING PROTOCOL STUDIES

Completed Protocol Type Studies

1.	NSC-4728	1,3,4-Thiadiazole, 2-amino-
2.	NSC-71795	Ellipticine
3.	NSC-79037	CCNU
4.	NSC-95441	Methyl CCNU
5.	NSC-104,801	Cytenbena
6.	NSC-118,994	Digylcolaldehyde, α-(hydroxy-methyl)-α'-(6-hydroxy-9H-purin-9-yl)-
7.	NSC-126,771	1,4-Napthoquinone, 2-(3,3-dichloroallyl)-3-hydroxy-
8.	NSC-132,313	Galactitol, 1,2:5,6-dianhydro-
9.	NSC-142,982	Hycanthone monomethanesulfonate
10.	NSC-145,668	Cyclocytidine
11.	NSC-157,365	Carzinostatin, crystalline antibiotic

Appendix

NSC-15200 Gallium Nitrate South Shore Analytical &Research Lab., Inc.

Preclinical toxicological evaluation of intravenously administered Gallium Nitrate was performed in dogs at the dose levels of 32 to 0.32 mg/kg and in monkeys at dose levels of 20 to 0.16 mg/kg. Animals demonstrated immediate but reversible prostration, apnea, mydriasis, bradycardia and salivation. Local irritation due to extravasated drug was indicated by perivascular hemorrhage, edema with fibrosis and necrosis noted at the site of drug injection. The effect of Gallium Nitrate on the blood and blood forming organs was mild and reversible. Decreases in erythroid elements and increases in leukocytes were observed with a pattern of marked increases in propertion of immature myeloid cell types. High doses of the agent produced lymphopenia in dogs but not monkeys. There was no evidence of methemoglobinemia in vivo. Histopathologic evaluation revealed various stages of lymphocytic degeneration and regeneration present in lymphoid tissues. Numerous foci of reticuloendothelial hyperplasia replaced the normal lymphoid follicular architecture, especially in areas of lymphoid depletion. Occasional areas of lymphoid hyperplasia exhibited numerous mitotic figures. Lethally intoxicated dogs demonstrated BSP retention which also was observed in a single monkey that died. Dose-related elevations of alkaline phosphatase, SGOT, SGPT and bilirubin were reversible. Abnormal histopathologic changes of the liver were observed only at lethal doses and consisted of cloudy swelling, hepatic focal necrosis and cholecystitis. Azotemia, electrolyte changes and elevated serum creatinine levels were dose-related. Albuminuria, glycosuria, hematuria and bilirubinuria were observed in animals receiving lethal doses. Histopathologic evaluation of the kidneys disclosed congestion, medullary hemorrhage and necrosis. At high doses, reversible changes in the eyes occurred and consisted of conjunctivities, corneal clouding and keratitis. The daily intravenous administration of the citrated solution of Gallium Nitrate to dogs at the dose levels of 10 mg/kg to 0.32 mg/kg for five consecutive days produced overt signs, hematologic and chemical changes, and pathological changes similar to those produced by equivalent doses of Gallium Nitrate. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-67574 Vincristine Battelle Memorial Institute

Preclinical toxicologic evaluation of intravenously administered Vincristine was performed in dogs at the dose levels of 0.08 mg/kg to 0.02 mg/kg and in monkeys at the doses of 0.64 mg/kg to 0.08 mg/kg. In both species, dose-related clinical signs of nervous system toxicity were seen and consisted of incoordination, loss of motor control and sensory innervation. Morphologic lesions consisting primarily of axonal swelling and neuronal degeneration were observed in both species but did not appear to be dose-related. There was a dose-related depression of red cell, white cell and to a lesser extent platelet counts. The white cell count was generally normal the day following injection, depressed four days later and returned to normal by the eighth day post injection. An associated reticulocytosis was observed. Clinical signs of digestive system toxicity were dose-related in both the dog and monkey. However, intestinal tract lesions in both species, consisting of villous atrophy,

superficial mucosal necrosis and inflammation of the lamina propria were observed over the entire range of doses. Dose-related elevations of SGOT were noted in the dog and monkey. On a mg/M^2 basis the dog and the monkey are more sensitive to Vincristine than man.

NSC-68075 Thalicarpine Arthur D. Little, Inc.

Thalicarpine was administered to the Rhesus monkey as a one hour infusion at doses ranging from 41.6 to 250 mg/kg x 1. Doses of 187.8 mg/kg and above were lethal. Death in monkeys was preceeded by convulsions, apnea, ventricular arrhythmia and coma. Doses below 83.6 mg/kg produced transitory depression of the white cell series, transitory irregular increases in BUN which were not strictly dose-related, transitory glycosuria or ketonuria and transitory elevations of transaminases. Administration of Thalicarpine by infusion greatly reduced the lethality of the drug in the monkey as compared to lethality following rapid intravenous injection. These data represented completion of the protocol study and have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-69856 Neocarzinostatin Mason Research Institute

The present preparation of Bristol Neocarzinostatin (Lot 71L 1052) is estimated to be approximately 10% pure as contrasted with the Japanese Neocarzinostatin (NSC-157,365) whose purity is sufficient to permit determination of its primary structure. Limited toxicologic studies were conducted by the intravenous route of administration in the Beagle dog at doses ranging from 20 mg/kg to 1.23 mg/kg. One of two dogs receiving weekly doses of 5 mg/kg went into anaphylactic shock when rechallenged with the drug. Precipitating antibodies were demonstrated in most subjects receiving high doses of the agent, even after single doses. The agent was also nephrotoxic; progressive uremia with hypercalcemia followed high single and repeated doses. Chronic interstitial nephritis was found on histopathological examination. Two dogs receiving high doses of the drug exhibited myocardial pathologic changes consistent with auricular infarction. In one case the EKG was consonant with the lesions found at necropsy. The leukopenia was severe; in the high dose dogs the marrow was frequently totally acellular and/or necrotic. Also observed were miscellaneous erratic effects such as pericholangitis, chalky necrosis of the abdominal fat, testicular degeneration and severe hemorrhagic enteritis.

NSC-71795 Ellipticine South Shore Analytical & Research Lab., Inc.

Ellipticine hydrochloride was administered as a 15 minute infusion to Beagle dogs at doses ranging from 32 mg/kg to 4.2 mg/kg. At all doses the agent as formulated was irritating to the veins used for injection. The leucocytosis sometimes seen in recipients of the drug three to five days after an infusion reflects a vigorous on-going phlebitic process. Occasionally a yellow deposit was seen at the injection site; this material may have been extravasated drug. Most of the affected veins did not repair, but thrombosed and sclerosed. Large single doses of Ellipticine and all repeated doses of the agent provoked

BSP retention and elevations of SGOT, SGPT, alkaline phosphatase and/or prothrombin time. These elevations were occasionally severe but were largely reversible. The sole histopathologic correlate of these clinical signs was a fatty metamorphosis of the liver of an occasional Beagle. Injections and to a far lesser degree infusion of Ellipticine depresses the blood pressure. The electrocardiographic arrythmias seen in an occasional dog may have resulted from transient hypotension. Large doses even when given by infusion produced ataxia, prostration, hyperventilation, tremors and convulsions. Occasionally neurologic sequellae in the form of sluggishness and hind-limb spread persisted for a day or two.

NSC-71851 9H-Purine-6-thiol, 2-amino-9-(2-deoxy-α-D-erythropento-furanolyl)-

α-TGdR

IIT Research Institute

α-TGdR was administered intravenously to dogs at doses of 300 to 6.25 mg/kg and to monkeys at doses of 600 to 18.75 mg/kg. Toxicity in both species is characterized by early development of emesis, diarrhea, anorexia, anemia and leukopenia followed by later development of thrombocytopenia and neutropenia. Lesions were often present at the injection sites and were caused at least in part by the high pH of the vehicle (pH 10.3). Vehicle toxicity prevented the escalation of the dose level to lethality in the single dose study in dogs and the daily for five days study in the monkeys. In the daily for 5 days study in dogs, the only study in which all four dose levels could be established, the slope of the dose-response curve is 32 (lethal dose/highest non-toxic dose) which is a very shallow slope for an oncolytic agent. The dose-response curve in the daily for 5 days study in monkeys is about 125. The primary reason for these shallow slopes is the fact that the hematological toxicity at the low doses does not increase proportionally as the doses are doubled. The toxicity of NSC-71851, αTGdR, can be compared to that of the β-anomer, β-TGdR, NSC-71261 and in the following table higher toxicity of β-TGdR in dogs is noted.

Dose	α-TGdR	β-TGdR
Single dose - LD	> 6000 mg/M^2	2800 mg/M^2
Single dose - HNTD	1500 mg/M^2	44 mg/M^2
Daily x 5 - LD	4000 mg/M^2/day	60 - 120 mg/M^2/day
Daily x 5 - HNTD	125 mg/M^2/day	7.5 mg/M^2/day

These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-77037 d-Tetrandrine-, (+)

Yale University School of Medicine

d-Tetrandrine was administered to dogs and monkeys intravenously at an infusion rate of 0.5 ml/minute (12.5 mg/minute) at dose levels of 100 to 10 mg/kg.

Initial investigations using intravenous injection (95 mg/minute) revealed development of an acute cardiovascular reaction manifested by cyanosis, ataxia, salivation, urination, defecation, hypotension, irregular bradycardia, and A-V dissociation frequently terminating with death. The acute post-injection cardiovascular reaction was circumvented in the majority of test animals by the slower rate of injection. Alteration of liver function parameters generally were reversible and occurred consistently regardless of the dose schedule. Histopathologic evaluation of liver sections revealed focal degeneration and necrosis, cholestasis, diffuse distribution of intra-hepatocyte lipofuscin granules and occasional multifocal granulomatous reactions. A mild reduction in circulating lymphocyte counts was correlated with a histopathologic finding of generalized necrosis of lymphoid germinal centers. A myelostimulatory-like effect was evidenced by an increase in circulating cells of the myeloid series and myeloid hyperplasia. Immature neutrophil counts were increased in the majority of test animals. Occasional increases in eosinophils correlated well with the findings of increased numbers of eosinophils in bone marrow smears. In addition, results of bone marrow differential counts revealed a mild increase in plasma cells. Mild anemia was evident at higher doses. Mild elevations in BUN levels were recorded occasionally with greater frequency in the monkey than in the dog. Only at high doses was the alteration correlated with histopathologic findings of corticotubular nephrosis. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-77213 Procarbazine Hydrochloride IIT Research Institute

Procarbazine was administered by the oral route to dogs at doses of 7.5 to 1.8 mg/kg and to monkeys at doses of 25 to 6.25 mg/kg. In both species, all blood cellular elements were affected although decreases in white blood cells appeared earlier and were more severe than changes in platelets or erythrocytes. Granulocytic changes were more marked in dogs whereas lymphocytes were more severely affected in monkeys. All changes were reversible in survivors, although only slowly in some animals. Severe effects were reflected in dose-related anorexia, emesis, diarrhea, enteritis and hemorrhages of the gastrointestinal tract. Both dogs and monkeys exhibited prolonged BSP retention and at lethal dose levels, elevated bilirubin levels. Elevation of SGPT was seen predominantly in dogs while BUN elevations were seen only in monkeys. Several dogs exhibited hypokalemia. Lethally intoxicated monkeys showed petechiae, ecchymoses or frank hemorrhage in a variety of organs suggesting altered capillary permeability. The male reproductive system appeared to be particularly susceptible as indicated by aspermia, aspermatogenesis and testicular atrophy.

NSC-79037 Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso- Mason Research Institute

CCNU

CCNU was infused in the Rhesus monkey over 20 minutes in a ethanol:propyleneglycol formulation at the dose of 20 to 2.5 mg/kg. The formulation was hemolytic: hemoglobinemia was proportional to the volume of vehicle

administered. Hemoglobinuria was seen in an occasional animal receiving the higher doses of drug, and therefore, of vehicle. Additionally, CCNU produced marked and protracted inhibition of erythropoiesis at high doses, as well as perceptible depressions of the white cell series at all doses studied, with leukopenic nadirs falling about 10 days after the single infusion. In monkeys, parenteral CCNU was markedly nephrotoxic. Animals receiving high doses became progressively uremic and at necropsy showed interstitial nephritis, with dose-related tubular dysplasia. Additionally, doses of 20 mg/kg produced progressive, severe hypercalcemia. The uremic state undoubtedly contributed to the protracted depression of erythropoiesis seen at the highest doses. Twenty minute infusions of CCNU produced striking dose-related lymphopenia and atrophy of the lymphoid tissues. Animals receiving 20 mg/kg exhibited hydropic degeneration of the liver. The clinical correlates of this histopathologic finding were: elevated transaminases, altered prothrombin times, and BSP retention. A significant elevation of serum bilirubin was seen only at the 20 mg/kg x 1 dose. CCNU was administered to the Beagle as a single doses ranging from 10 to 0.625 mg/kg. Doses below 2.5 mg/kg depressed the white blood cells, but not to the point of leukopenia. All doses of the drug, but not the vehicle, produced a delayed monocytosis. Chemical signs of prompt hepatic dysfunction were seen above 0.625 mg/kg doses. These included retention of BSP and increased alkaline phosphatase levels. At post-mortem examination, pathologic changes of the liver were seen only at the highest dose and consisted solely of congestion. Atrophy of lymphoid tissues and thymus was seen only after a single injection of 10 mg/kg. The highest dose produced leukopenia which resulted in florid cellulitis of the paws.

NSC-82151 Daunomycin International Research & Development Corp.

The clinical formulation of Daunomycin was administered intravenously to dogs and monkeys on a schedule of a daily treatment for 3 days followed by 3 days of no treatment. The schedule was repeated 5 times for a total of 15 treatments when the dogs survived. A dose-related adverse effect on all blood cell types was observed. Marked atrophy and hemorrhage of lymphoid tissues were observed at doses of 1 mg/kg/day and greater. Except for hemorrhages in the heart at the highest doses, there were no cardiovascular lesions. The primary organs which were adversely affected in the monkey were of the hematopoietic and lymphoid systems. At the highest dose (2 mg/kg) there were lesions in the intestinal tract of all monkeys. There were transient changes in many of the blood chemical parameters (magnesium, blood sugar, BSP, calcium, creatinine, sodium chloride) at all dose levels. These changes were fewer in number and less severe at the lowest dose (0.5 mg/kg). The Lethal Dose/Highest Non-Toxic Dose is 4, a moderately steep dose-response curve.

NSC-83265 L-Alanine, 3-(tritylthio)- (Trityl cysteine) Arthur D. Little, Inc.

Administration of single intravenous doses (100 - 25 mg/kg) of trityl cysteine to Beagle dogs produced dose-related toxic alterations. Acute effects included emesis, incontinence, and terminal apnea. Lethargy, anorexia, and weight loss were also noted. These overt signs and the hepatic, renal, and myelopoietic toxicities are qualitatively consistent with those seen in earlier oral toxicity

studies. The intravenous lethal dose is approximately one-twentieth that following oral administration. Reversible elevation of serum transaminases and alkaline phosphatase were seen following all toxic dose levels of trityl-cysteine. One lethally intoxicated dog exhibited hyperbilirubinemia and one dog at the 100 mg/kg dose showed reversible BSP retention. Histopathologic evaluation of hepatic tissues revealed congestion, glycogen reduction and cloudy swelling. Bilirubinuria and hematuria were seen at doses above 50 mg/kg and azotemia at the lethal level only. The 100 mg/kg dose produced glucosuria in one dog. The only histopathologic evidence of toxicity was moderate glomerular capillary congestion. Death of one dog on day 4 was preceded by severe leukopenia. Lymphoid depletion was apparent in the spleen; the bone marrow exhibited myeloid hypoplasia. Following administration of repeated intravenous doses of 50 - 6.25 mg/kg to Beagle dogs and of 50 - 0.78 mg/kg to Rhesus monkeys, abnormal clinical signs were observed only at high doses. These included anorexia, weight loss, retching or emesis, dyspnea, bradycardia, and lethargy. Hematologic and clinical chemical observations reflect effects on the liver and hematopoietic organs in both species and, particularly in monkeys, the kidneys. All effects were reversible. Elevation of SGPT, SGOT and alkaline phosphatase were seen in both dogs and monkeys. Marked elevation of serum bilirubin levels was noted only in monkeys. These changes were dose-related in severity and reversible. Hepatic histopathologic changes were conspicuous in animals sacrificed immediately after dosing, but were minimal in those sacrificed on day 50. In monkeys, parenchymal damage appeared less severe than in dogs, but bilirubin elevation was associated with jaundice, biliary stasis and degeneration of the bile duct epithelium. No clinical signs of renal toxicity were noted in dogs. Histopathologic signs seen in one dog which died included swelling of the epithelial cells of the glomerular capillaries and increased lipid in the tubular epithelium. In monkeys, renal involvement is indicated by progressive BUN elevation, increases in serum creatinine, and electrolyte abnormalities. Histologic changes included ulceration and necrosis of the epithelium of the kidney, and ureters, and hemorrhagic cystitis. In dogs, marked leukopenia, confirmed by detection of myeloid depression in the bone marrow, lymphoid hypoplasia in the lymph nodes and spleen were observed only in one animal which succumbed. Drug-related hematologic changes in monkeys included moderate anemia, marrow hypoplasia and lymphoid depletion in the spleen, lymph nodes and thymus. Microfocal necrosis and exfoliation of the epithelium of the tonsils, buccal cavity, tongue, and colon were more conspicuous in dogs than monkeys. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-95441 Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso- Mason Research Institute

Methyl CCNU

Methyl CCNU was given intravenously to Beagle dogs at dose levels of 14 to 1.56 mg/kg in an intralipid-ethanol formulation. This formulation of Methyl CCNU was not altogether free of hemolytic action. Life-threatening myeloid hypoplasia was produced by the single injection of 14 mg/kg. Animals died of the consequences of sepsis. All doses above 3.12 mg/kg exerted some

depressive influence on the marrow - especially on its myeloid elements. Only at the uppermost doses was hepatotoxicity evident: BSP retention, increased alkaline phosphatase, and increased prothrombin time all pointed to hepatic dysfunction. One animal at the dose of 14 mg/kg x 1 died in sepsis with uremia. When MeCCNU was extravasated accidentally, a slowly developing necrotic condition developed at the site of leakage; this process eventually crippled the affected limb. Many dogs at all doses - along with the vehicle control - exhibited bloody diarrhea at some point in the study. It is not impossible that an intercurrent enteritis was causative of this effect. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

Methyl CCNU (NSC-95441), given to Beagles as a single intravenous infusion in 20% ethanol: 80% propylene glycol, was hemolytic at all doses (14 - 1.56 mg/kg) studied - i.e. The vehicle alone was hemolytic in the eight Beagles tested. Toxicity, typical of the nitrosoureas was produced in dogs. The highest doses studied was profoundly and promptly myelotoxic with neutropenia becoming evident by day 4 and progressing to day 10. Sepsis was a sequel to this toxicity. Doses below 9 mg/kg produced only very mild myelotoxicity. Monocytosis was seen during recovery from the myelosuppressive actions of the drug. At the highest dose, atrophy, necrosis and hemorrhage of lymph nodes and/or tonsils was a salient pathologic finding; both dogs receiving this dose also were lymphopenic. BSP retention, elevated transaminases, prolonged prothrombin times and major elevations of alkaline phosphatase were seen only at the top dose of 14 mg/kg x 1. One of the animals receiving the highest dose exhibited focal vacuolization of the epithelium of the urinary bladder - while the companion exhibited peculiar focal epithelial hyperplasia of both epididimides. In addition to diffuse petechiae and hemorrhages, the cardiac valves of both dogs at the highest-dose were damaged. One of these lesions was pronounced incidental, the other, a myxomatous degeneration of the pulmonic valve, could have been drug-induced. This ethanol-propylene glycol formulation is more hemolytic than the "Intralipid" formulation. Extravasation of both formulations should be carefully avoided. Drug-effects were very largely independent of the formulation used. Since the experimental subjects were sacrificed on or about day 50, truly delayed toxicity to liver or kidney might have escaped detection. Methyl CCNU was given as a single intravenous infusion to Rhesus monkeys in a propylene glycol:ethanol formulation. The highest dose, 30 mg/kg x 1, produced severe depression of granulopoiesis and erythropoiesis. During recovery from this toxicity, monocytosis and eosinophilia were detected. One out of three monkeys at the highest dose developed progressive uremia. While the animal was sacrificed in good general condition, on day 100, the lesion was felt to be a lethal one. Some frank elevation of SGOT was seen at the highest dose and a tendency towards elevation at the two lower doses. These changes probably reflect a mild hepatotoxic action of the drug.

NSC-102,816 5-Azacytidine Arthur D. Little, Inc.

5-Azacytidine formulation with polyvinylpyrrolidone (PVP) was applied topically to the hamster cheek pouch and given intravenously to the hamster to study its effects on micro-circulation. Decreased blood flow in arterioles and

constriction were observed after topical and intravenous administration. Administration of PVP alone produced decreased venule flow but only after doses twenty times those in the 5-azacytidine formulations. No meaningful alterations were observed in clinical signs, hematologic, and clinical chemical tests, or pathologic studies which suggest toxic effects attributable to PVP.

NSC-112,907 Tetrahydrouridine International Research & Development Corp.

No dose-related toxic changes were present in the Beagle dogs treated with single intravenous injections of Tetrahydrouridine at the doses of 1000 - 250 mg/kg. Although there were sporadic alterations of parameters such as reticulocyte and platelet counts, and serum magnesium, chloride, and sodium levels these did not appear to be a direct consequence of toxicity of the drug. The drug did appear to cause necrosis at the site of injection. In the Rhesus single intravenous doses of 1000 - 250 mg/kg caused a consistent mild to moderate non-dose-related depression of the erythroid elements of the hematic system. There was also a consistent mild rise in the SGOT in the first two days after treatment.

NSC-119,875 Platinum, diamminedichloro-, *cis*- Mason Research Institute

In the present study, four Rhesus monkeys were given the Toxic Dose Low (TDL) 1.25 mg/kg of NSC-119,875, daily for 5 days. Two vehicle controls received distilled water only. The drug produced very prompt increase in 24 hour sodium and potassium excretion, early increases in 24 hour LDH excretion in the urine, transient modest but persistent increases in BUN and questionable transient increase in creatinine, which was often but not always synchronous with the increased BUN levels. As a consequence of sodium retention, the tubular epithelial cells imbibe water, swell and become leaky with respect to LDH. On the basis of these changes, the pathogenesis of Platinum nephrotoxicity can be reconstructed as follows: As few as two doses (1.25 mg/kg) derange tubular function and impair the net excretion both of sodium and potassium but not to such a degree that urinary specific gravity or refractive index are altered. Intraluminal pressures sporadically increase as a consequence of the swollen epithelium and glomerular filtration of BUN and creatinine. Thus, the uremia seen was intermittent and reversible as opposed to progressive. In a previous study when monkeys were given 1.25 mg/kg of NSC-119,875 for 5 days, protreacted polyuria resulted. This phenomenon serves to confirm the conclusion that Platinum damages the renal tubules. This pilot study confirms the utility of using urinary enzymes as indicators of renal toxicity. Careful assessment of electrolyte balance would also appear to be an early index of tubular dysfunction in the case of NSC-119,875.

Cis-dichlorodiamino platinum was tested for ototoxic effects in guinea pigs. Ototoxicity was evaluated by the disappearance of Preyer's reflex in response to pure tones of 5, 7 and 10 kHz and by histopathological evaluation of the inner ear. Treatment with a single intraperitoneal dose produced no specific

ototoxicity whereas repeated daily treatments with 1 mg/kg five times a week until hearing loss occurred led to deafness in all animals without incidence of mortality. Vestibular toxicity was not observed. Phase contrast microscopy of surface preparations from the organ of Corti revealed loss of outer hair cells primarily in the basal turn of the cochlea. Hair cell lesions included degeneration, complete cytolysis and replacement with phalangeal scars. The platinum-induced lesions resembled those produced by other ototoxic agents except that inner hair cell lesions occurred less frequently. In the rat, single intraperitoneal injection of 7 to 15 mg/kg produced dose-related mortality and hearing loss to a tone of 10 kHz in one of the rats treated with 9 mg/kg. Comparison of the rats for hearing loss and mortality indicates that the rat is less susceptible to ototoxicity than the guinea pig.

Mice (Charles River CD1) four to six weeks old were treated with single ip dose of 13.9 mg/kg of the platinum agent. On day 2, BUN levels were slightly elevated and proteinuria and glycosuria were observed although no renal tubular necrosis was apparent. By day 3, females showed severe proteinuria, which correlated with an earlier onset of renal tubular necrosis in females than was observed in the male mice. By day 4, the onset of renal tubular necrosis in males correlated with severe proteinuria. Slight but progressive hypercalcemia and hypokalemia were also observed and may be related to renal toxicity. Review of the kidneys from platinum-treated mice sacrificed three to seventeen days following a single intraperitoneal graded doses of 16.7, 13.9 or 11.6 mg/kg revealed essentially dose time and sex related renal pathologic lesions. The incidence of mortality was strikingly higher in females (11/36) than in the male (0/36). Lesions observed included focal tubular necrosis, eosinophilic casts, dilation of tubules and in some cases focal tubular dysplasia and regeneration. Tubular necrosis was apparent as early as day 3 in all animals and could be detected as late as day 13 in some animals. By day 17, no tubular necrosis was apparent and complete regeneration had occurred with no obvious residual lesions although the blood urea nitrogen was still elevated in a few animals. The earliest evidence of tubular regeneration occurred on day 11. In general the blood urea nitrogen levels correlated well with the extent of renal pathology although there were a few animals displaying renal tubular necrosis and normal blood urea nitrogen.

NSC-121,210E Androst-5-en-17-one, 3β-hydroxy-, [p-[bis(2-chloroethyl)amino]phenyl] acetate

DHEA Mustard

Mason Research Institute

A total of 4 of the 26 treated monkeys at doses levels of 300 to 1.35 mg/kg died from intestinal or renal lesions associated in part with presumed activation of latent Shigellosis. Control monkeys receiving oral vehicle (sesame oil) exhibited anorexia, weight loss, and alterations in hematologic and blood chemistry parameters. In general, the toxicologic findings with DHEA mustard administration to monkeys were similar to those reported previously in the dog with the exception of effects upon reproductive organs.

The monkeys did not exhibit persistent histopathologic alterations in testes, uterus, and mammary glands as reported in the dog. The findings of frequent lymphopenia correlated well with the histopathologic evidence of generalized lymphoid atrophy. In addition, leukopenia, reticulocytosis, immature netrophilia, and thrombocytopenia were associated with varying degrees of bone marrow atrophy. The anemia observed was most likely related to frequent blood collection procedures. Gastrointestinal toxicity was manifested overtly by anorexia, emesis, diarrhea, weight loss, and dehydration. Occasional alterations of liver function parameters were correlated with histopathologic findings of hepatic fatty metamorphosis. Alterations of serum electrolytes may be related to loss of electrolytes due to emesis and diarrhea. Frequent elevations of BUN levels were associated histopathologically with findings of lipoid nephrosis, proteinaceous tubular casts, and tubular fatty metamorphosis. These data are adquate for INDA filing.

NSC-125,066 Bleomycin Mason Research Institute

Bleomycin was administered to the Beagle dog at the dose of 5 mg/kg every fourth day as a single injection or fourteen injections. Severe pulmonary fibrosis often affecting large areas of the total lung surface was observed; in addition to this desmoplasia, there was evidence of interstitial pneumonitis, pulmonic small-vessel perivasculitis and bronchiolitis - despite the fact that the animals were sacrificed approximately 60 days after the last treatment. All the treated subjects had just become floridly uremic or were on the verge of so becoming at the time of sacrifice. Thus, the manifestations of renal failure were delayed. Dermatologic signs, typical of Bleomycin, were observed and were reversible. Also noted were depressed levels of zinc in plasma, which reverted to within normal limits when Bleomycin was stopped. There was also an indication of depression of zinc in the lung. Despite the severity and chronicity of the lesions in these subjects, the erythrocyte sedimentation rate was not greatly accelerated. Moreover lupus erythematosus preparations remained negative throughout the study.

NSC-135,758 2,5-Piperazinedione, 3,6-bis-(5-chloro-2-piperidyl)-dihydrochloride Battelle Memorial Institute

Preclinical toxicological evaluation of this agent was performed using the intravenous route in dogs at the dose levels of 3 to 0.0937 mg/kg and in monkeys at 1.5 to 0.0937 mg/kg. There was a consistent dose-related adverse effect of NSC-135,758 on the hematic system. The granulocytic, erythrocytic, and thrombocytic series were all affected with the granulocytic series manifesting the most damage. These changes were reversible in the surviving animals. A consistent dose-related adverse effect on the lymphoid system also was present in both dogs and monkeys. The leukopenia consisted of a decrease in both the lymphocytic and granulocytic elements. Morphologically there was lymphoid depletion of the lymphoid organs. Hemorrhages, necrosis and villous atrophy occurred in the intestines in a non-dose related manner. Hemorrhage, edema, congestion and bacterial invasion of the lungs were present at the higher dose levels. Testicular degeneration and atrophy occurred in

dogs in a non-dose related fashion. NSC-135,758 is quite toxic but the toxic changes appear to be manageable. The slope of the dose-response curve (LD/HNTD) for dogs with a single injection is 4, a steep dose-response. For the daily x 5 schedule in dogs the slope is 8. For the daily x 5 schedule in monkeys the slope is 32, a much less steep slope. It can be noted however that the HNTD is in the same range (in mg/M^2) for both dogs and monkeys for the daily x 5 schedule but that the LD for monkeys is higher. When the TDH from the single dose study was administered to dogs once a week for 6 weeks there was some increased toxicity but no lethality. When the TDH from the daily x 5 dose study in dogs was administered to dogs for 3 five day periods each separated by 9 days, there was no increased toxicity. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-136,020E Androst-4-en-3-one, 17β-hydroxy-7β-methyl-, propionate — Mason Research Institute

Preclinical toxicological evaluation of 7β-methyl testosterone propionate was performed in two groups of four dogs receiving intramuscular doses of 50 or 25 mg/kg for 28 consecutive daily doses. An additional two dogs were used for vehicle controls. Ancillary studies in rabbits receiving 83 mg/kg of the agent for five consecutive days revealed elevated BSP retention in 2/6 animals. No significant gross or microscopic hepatic lesions were observed in any of the treated rabbits. A total of 10 rats were treated intramuscularly with single doses of 1000, 320 or 100 mg/kg with no adverse clinical effects evident. Body weight gain was significantly greater in treated female rats than in non-treated female controls. Gross alterations at the injection site included swelling and ulceration. Histopathologic evaluation of areas at the site of intramuscular injection revealed hemorrhage, necrosis, edema, inflammation, muscular atrophy, and accumulation of fat globules. Elevations in SGPT and SGOT were not correlated with any significant hepatic morphologic changes. Regional lymph nodes in the area of intramuscular injection sites contained hemorrhage, accumulation of fat globules and foci of inflammation and fibrosis. A transient lymphocyte decrease was evident in both treated and control dogs. Fluctuations in total white blood cell count and hyperplastic bone marrow alterations in treated and control dogs may be related to chronic focal infectious processes at multiple intramuscular injection sites. Endometrial and mammary hyperplasia was evident in two of four treated female dogs; one at each dose level. Fat globules were observed occasionally in sections of kidneys and lungs. Although a number of the above alterations are most likely related to the type of vehicle utilized the majority of changes observed would be consistent with an agent having androgenic activity. These data are adequate for INDA filing.

NSC-139,105 Ethanesulfonic acid, compd. with α-[-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenoxy]-N,N-dimethyl-m-toluamide (1:1)

Baker's Antifol — Yale University School of Medicine

Baker's Antifol was administered intravenously on various schedules at doses ranging from 0.3125 to 40 mg/kg to Beagle dogs and Rhesus monkeys. Drug related deaths were produced in 4 dogs and one monkey. The following is a summary of the major organ system toxicity observed in the dogs and monkeys. An acute post injection reaction consisting of tachycardia, tachypnea, dyspnea, ataxia and occasional respiratory arrest was observed at the higher doses. The clinical signs of anorexia, weight loss, emesis and hemorrhagic diarrhea correlated well with histopathologic findings of diffuse hemorrhagic necrotizing enteritis. Altered liver function tests, which occurred occasionally, were consistent with hepatic congestion, cellular degeneration and fatty metamorphosis. Lymphocyte counts in the peripheral blood fluctuated considerably whereas lymphoid depletion was a consistent histopathologic finding in spleen, thymus, lymph nodes and tonsils. Frequent hematologic alterations consisted of mild reduction in WBC counts, hemoconcentration, increase in immature neutrophils, eosinophils, and reticulocytes. Evaluation of bone marrow smears revealed hypocellularity with depletion of both myeloid and erythroid elements. Alterations in renal function consisted of elevations of blood urea nitrogen and creatinine levels. Urinalysis revealed increased amounts of protein, and presence of white blood cells, red blood cells and crystals. Histopathologic evaluation of kidney sections resulted in essentially negative findings. Therefore alteration in kidney function may be related to a secondary response to dehydration rather than the result of a drug-related nephrotoxic effect. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

NSC-143,769 Methanol extraction residue (MER) of BCG — Mason Research Institute

MER-BCG

MER-BCG is the methanol insoluble residue of phenol-killed, acetone washed BCG (Bacillus Calmette-Guerin). It has been demonstrated that MER-BCG enchanced the immunologic capacity and resistance to experimentally induced tumors, and decreased the incidence of spontaneous tumors. In dogs, intradermal injections of MER-BCG, 0.1 mg in 0.1 ml of steroid suspending vehicle, led to granuloma formation. The lesions reached approximately 10 mm in diameter, ulcerated and were accompanied by granulomatous changes in the regional lymph nodes. Treatments with 10 daily injections of 0.1 mg for one or six weekly treatments failed to produce any other histopathological changes, or changes in physical condition, hematological and blood chemical parameters. The immunogenicity of MER-BCG was characterized by a potent cell-mediated immune response (non-specific host resistance enchancing activity, positive tuberculin reaction and granuloma formation). A humoral response to the administration of MER-BCG was not definitely established. These data have been submitted to the Cancer Therapy Evaluation Branch for INDA filing.

Emulphor (EL-620) — Mason Research Institute

Emulphor (EL-620), a possible vehicle for I.V. infusion of CCNU (NSC-79037) and Methyl CCNU (NSC-95441), was tested for blood pressure changes to unanesthetized dogs and monkeys. Treatments were performed with saline

containing 5% Emulphor and 5% Ethanol. The rapid I.V. injection of 10 mg/kg Emulphor (0.2 ml/kg) produced a severe decrease of blood pressure, with bradycardia and severe sinus arrhythmia. The infusion of 187.5 mg/kg Emulphor (3.75 ml/kg , within approximately 60 minutes) produced a similar drop in blood pressure. Fifteen to 30 minutes after onset of treatments there also occurred erythema and edema of the ears, injection of the conjunctivae and hyperemia of the eyelids, which lasted approximately 60 minutes. Pretreatment with the above rapid I.V. injection as a priming dose, reduced the blood pressure effects of the subsequent Emulphor infusion. The two monkeys that were infused intravenously for 60 minutes with Emulphor 666.0 mg/kg (13.3 ml/kg) showed a slight decrease of blood pressure, which was less than 20 mm Hg, without any other toxic manifestations.

SUMMARY REPORT

LABORATORY OF TUMOR CELL BIOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1972 -- June 30, 1973

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification and determination of viral information in human leukemic cells; (3) cell cycle characteristics; (4) search for biochemical markers of minimal neoplastic disease; (5) biochemical and physiological studies on leukocyte maturation in vitro. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

During the past year a number of findings were reported by investigators from the laboratory:

1. Purification of sufficient DNA polymerase from human leukemic cells to allow an immunologic demonstration of similarity to reverse transcriptases of simian RNA tumor viruses.

2. Purification and characterization of DNA polymerases from normal human lymphocytes.

3. Purification and characterization of reverse transcriptases of simian virus origin.

4. Development of a methodology for determining the origin of candidate human tumor viruses.

5. Demonstration of the target cell for murine leukemia virus in the murine hemopoietic organs.

6. Identification of lymphocytes as a quantitatively important source of maturation factor for leukocytes.

7. Characterization of a human cell line which matures in vitro into granulocytes which promises to allow the first detailed description of human leukocyte differentiation from a molecular biological point of view.

8. Development of a procedure for purifying human myeloid colony-stimulating factor.

9. The finding that the RNA of all RNA tumor viruses (avian to primate) contains a poly A, and this poly A is homogeneous, and is about 200 nucleotides in length. This provides: (a) a new criterion for demonstrating if an RNA in human tumor cells is indeed viral; (b) a new chemical assay for these viruses; (c) more information on their life style.

10. Demonstration that cordycepin, an inhibitor of poly A synthesis, blocks the expression of RNA tumor virus genetic information in mouse cells.

11. Demonstration that certain derivatives of rifamycin SV effectively prevent viral leukemogenesis in mice when the virus is pre-treated with such compounds. This is a natural in vivo extension of previous work in which we showed similar results in vitro.

12. The finding (along with other laboratories) of specific tRNA species within RNA tumor viruses and the characterization of these tRNA's.

13. The purification and characterization of hybridase (Ribonuclease H) form mammalian RNA tumor viruses and form human lymphocytes and the finding of two inhibitors of this activity.

14. The analysis of the DNA polymerase inhibiting effects of ara-CTP (collaboration with Dr. Anthony Schrecker).

15. The demonstration that the nucleic acid information of DNA tumor viruses is not related (within limits of detection) to the information of RNA tumor viruses (molecular hybridization studies).

16. The demonstration that poly U inhibits viral reverse transcriptase but not preferentially since it inhibits one of the major normal cellular DNA polymerases.

17. The demonstration that certain derivatives and modifications of thymidylate are potent but not selective inhibitors of viral reverse transcriptase. This was in collaboration with Dr. Anthony Schrecker.

18. The demonstration that RD114, a candidate human tumor virus, is, in fact, a cat virus.

19. The development of a new micro technique of molecular hybridization which will allow experiments to be done not previously possible.

20. Immunological evidence that the reverse transcriptase we isolated from leukemic cells is viral in origin (collaboration with Dr. George Todaro).

21. Finding of antibodies to RNA-DNA hybrids in the sera of patients with systemic lupus erythematosis (collaboration with Dr. Norman Talal).

22. The development of a new improved technique for the isolation of RNA-DNA hybrid products of early reactions catalyzed by reverse transcriptase.

Serial No. NCI-6117
1. Laboratory of Tumor Cell Biology
2. Section on Cellular Control Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Mechanisms Leading to Neoplastic Transformation; On Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells; On New Approaches to Cancer Chemotherapy; and On the Development of Biochemical Markers of Neoplastic Cells.

Previous Serial Number: SAME

Principal Investigator: Robert C. Gallo

Other Investigators: Robert Gallagher, R. Graham Smith, Prem Sarin, W. Carl Saxinger, Brian Lewis, David Miller, Robert Mayer, Jacqueline Whang-Peng, Michael Paran, Marjorie Robert, Paul Gerber, Richard Adamson, Dave Gillespie, Michael Sporn, Tony Schrecker, Robert Suhadolnik, Lawrence Loeb, George Todaro, Jeff Schlom, Umberto Torelli, Leon Dmochowski, Sol Spiegelman, Fritz Deinhardt, Robert Nowinski, Norman Talal, and Art Levine

Cooperating Units: Laboratory of Viral Immunology, Division of Biologics Standards; Laboratory of Chemical Pharmacology, Carcinogenesis, NCI; Albert Einstein Medical Center; The Institute for Cancer Research; Columbia University College of Physicians and Surgeons; University of Modena; M.D. Anderson Hospital and Tumor Institute; McArdle Laboratories; and Veterans Administration Hospital.

Man Years:

Total: 16 1/4
Professional: 10
Other: 6 1/4

Project Description:

Objectives:

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

   a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

   b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformation?

   c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

   Several of the above questions have been answered at least in part.

2. Chemical studies of oncornavirus specific RNA and on the overall pathway of replication of these viruses. Purposes:

   a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

   c. In understanding the mechanisms involved in expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference.

3. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up following the techniques of Paran and Sachs (Paran is now in our laboratory). Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Characterization of tRNA species present in RNA tumor viruses.

5. Developing methods for getting specific RNA species into animal cells intact and to determine their biological effects.

6. Development of potent and selective DNA polymerase inhibitors to be evaluated on fresh human acute leukemic cells and proliferating normal lymphocytes in short term culture.

Methods Employed:

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72, 1972; Proc. Natl. Acad. Sci. 69: 2879-2884, 1972; Proc. Natl. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972, in press).

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. Product analysis of DNA synthesized by various DNA polymerases is carried out by conventional techniques including:

   a. "Nearest neighbor" analysis.

   b. Size estimate and nature (DNA-RNA or DNA) were made by gel electrophoresis and by glycerol gradient ultracentrifugation untreated, heat-treated, RNase and/or alkali treated.

c. Complementarity by three different molecular hybridization techniques: Cs_2SO_4 density gradient equilibrium centrifugation (liquid), specific nucleases, and millipore filter.

6. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs.

7. The characterization of tRNA species (J. Mol. Biol. 52: 195-219, 1970) have been previously described.

8. Procedures for uptake of RNA into animal cells has been described (Proc. Nat. Acad. Sci. 67: 1943-1950, 1970). The demonstration of biological function of these RNA molecules added from one cell to another was evaluated as described (Biochem. Biophys. Res. Comm. 49: 782-792, 1972).

9. Induction of type C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

Major Findings:

1. In E. coli only one DNA polymerase was known for many years, in the last few years two other DNA polymerases were discovered. Our laboratory has isolated and partially purified three DNA polymerases from normal and leukemic human lymphocytes. One polymerase can catalyze DNA synthesis from an RNA template. This was the first finding of this type of enzyme in any animal cell. Template and other biochemical properties gave support to the possibility that this enzyme may have been viral derived. For example, the enzyme from leukemic cells will copy the genomic 70S RNA from avian RNA tumor viruses. The DNA made is complementary specifically to the template viral RNA, i.e., true reverse transcriptase. No DNA polymerase from normal human lymphocytes has this property. In fact, to date there is no evidence in any normal cell for an enzyme which copies a single-stranded natural RNA. Most importantly, the major discovery made in this laboratory during the past year, was the demonstration that reverse transcriptase from human leukemic cells was immunologically identical to primate (gibbon ape) type C leukemia virus reverse transcriptase. This study was done in close collaboration with Dr. George Todaro of NCI.

   These observations, in our judgment, are the best available evidence that at least some human leukemic cells contain a virus specific protein, namely reverse transcriptase. This does not prove that this information is causatively related to the disease, but obviously combined with evidence from animal systems these observations markedly increase this suspicion. The task now will be to determine the distribution (% of patients) with this enzyme, whether it is correlated with a particular stage of the disease, and whether it is essential for persistence of the leukemic state.

The reciprocal immunological experiments (i.e., antibodies developed against normal human cellular DNA polymerases and to the human leukemic reverse transcriptase tested against the human cellular and viral polymerases) will be of great interest and are now in progress.

2. Reverse transcriptase from two recently isolated primate RNA tumor viruses, Mason Pfizer Monkey virus (MPMV) and the Simian Sarcoma virus (SSV-1), were purified to apparent homogeneity. The purified enzymes exhibited properties thought to be common for all viral reverse transcriptases, yet the results of these studies brought out important heterogeneity of these viral polymerases even when derived from the same species, e.g., important differences in cation requirements and molecular weight between these two primate viruses were found.

   Plans with this portion of our studies will be: (a) development of antibodies to these enzymes; (b) study the properties of these enzymes when derived from cells (i.e., intracellular virus rather than released virus).

3. The mechanism of poly U inhibition of viral reverse transcriptase was further investigated. We observed that although this is a very potent inhibitor of the viral polymerase, it lacks specificity. Modifications of poly U were made by Miles Laboratory, and the derivatives are now under study. If no specificity is found this study will be terminated.

4. Purification and characterization of DNA polymerases from normal human lymphocytes.

5. Development of a methodology for determining the origin of candidate human tumor viruses.

6. Demonstration of the target cell for murine leukemia virus in the murine hemopoietic organs.

7. Identification of lymphocytes as a quantitatively important source of maturation factor for leukocytes.

8. Characterization of a human cell line which matures in vitro into granulocytes which promises to allow the first detailed description of human leukocyte differentiation from a molecular biological point of view.

9. Development of a procedure for purifying human myeloid colony-stimulating factor.

10. The finding that the RNA of all RNA tumor viruses (avian to primate) contains a poly A, and this poly A is homogeneous, and is about 200 nucleotides in length. This provides: (a) a new criterion for demonstrating if an RNA in human tumor cells is indeed viral; (b) a new chemical assay for these viruses; (c) more information on their life style.

11. Demonstration that cordycepin, an inhibitor of poly A synthesis, blocks the expression of RNA tumor virus genetic information in mouse cells.

12. Demonstration that certain derivatives of rifamycin SV effectively prevent viral leukemogenesis in mice when the virus is pre-treated with such compounds. This is a natural in vivo extension of previous work in which we showed similar results in vitro.

13. The finding (along with other laboratories) of specific tRNA species within RNA tumor viruses and the characterization of these tRNA's.

14. The purification and characterization of hybridase (Ribonuclease H) form mammalian RNA tumor viruses and form human lymphocytes and the finding of two inhibitors of this activity.

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16. The demonstration that the nucleic acid information of DNA tumor viruses is not related (within limits of detection) to the information of RNA tumor viruses (molecular hybridization studies).

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19. The demonstration that RD114, a candidate human tumor virus, is, in fact, a cat virus.

20. The development of a new micro technique of molecular hybridization which will allow experiments to be done not previously possible.

21. Finding of antibodies to RNA-DNA hybrids in the sera of patients with systemic lupus erythematosis (collaboration with Dr. Norman Talal).

22. The development of a new improved technique for the isolation of RNA-DNA hybrid products of early reactions catalyzed by reverse transcriptase.

Significance to Biomedical Research and the Program of the Institute:

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved appraches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course:

As described above, some projects will terminate and others will continue to be actively pursued.

Honors and Awards:

1. "Cancer Prophylaxis and Remission Maintenance Concepts Derived From Studies on Viral Oncogenesis." Presented at the Fifth International Congress on Pharmacology, San Francisco, California. July 1972.

2. "RNA Directed and Primed DNA Polymerase Activities in Tumor Viruses and Human Lymphocytes." Presented at the 2nd Annual Steenbock Symposium, Madison, Wisconsin. July 1972.

3. "RNA Tumor Viruses, Reverse Transcriptase, and Human Leukemia." Presented at the Symposium on Polyamines: Their Implications for Effective Cancer Regulation, Hunt Valley, Maryland. October 1972.

4. "On the Nature of the Nucleic Acids and RNA Dependent DNA polymerase from RNA Tumor Viruses and Human Cells." Presented at the IV Lepetit Colloquium on Possible Episomes in Eukaryotes, Cocoyoc, Mexico. November 1972.

5. "Origin of RNA Tumor Virus Genomes." Presented at the Meeting of the American Federation for Clinical Research, Atlantic City, New Jersey. April 1973.

6. "Reverse Transcriptases." Presented at the Workshop on Cellular and Viral DNA Polymerases, Deauville, France. June 1973.

7. "What is the Evidence for a Human Leukemia Virus?" Presented at the 8th Congress of the Nordic Society for Cell Biology, Tromso, Norway. June 1973.

8. "Molecular Biological Studies on Human Leukemogenesis." Presented at the Workshop on Modern Trends in Human Leukemia, Hamburg, West Germany. June 1973.

9. "A Summary of Recent Observations in the Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Cancer." Presented at the Fels Research Institute, Temple University, Philadelphia, Pennsylvania. September 1972.

10. "I. Reverse Transcriptase of RNA Tumor Viruses and Human Leukemic Cells. II. Recent Observations on the Induction of Type C Viruses." Presented at the University of Pennsylvania, Department of Microbiology, Philadelphia, Pennsylvania. November 1972.

11. "A Summary of Recent Observations in the Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Cancer." Presented at the University of Colorado, Department of Microbiology, Denver, Colorado. November 1972.

12. "A Summary of Recent Observations in the Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Cancer." Presented at the Yale University School of Medicine, Department of Pharmacology, New Haven, Connecticut. November 1972.

13. "Observations on the Molecular Biology of RNA Tumor Viruses." Presented at the Children's Hospital Center, Boston, Massachusetts. November 1972.

14. "The Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Leukemia." Department of Pathology, Jefferson Medical College, Philadelphia, Pennsylvania. December 1972.

15. "Observations on the Molecular Biology of RNA Tumor Viruses." Presented at the Institute for Cancer Research, Philadelphia, Pennsylvania. March 1973.

16. "The Molecular Biology of RNA Tumor Viruses and Attempts at Application to Human Leukemia." Presented at the Department of Medical Microbiology and Immunology, University of California at Los Angeles. March 1973.

Publications:

1. Ting, R.C., Yang, S. S., and Gallo, R. C.: Reverse Transcriptase, RNA Tumor Virus Transformation and Derivatives of Rifamycin SV. Nature New Biology 236: 163-166, 1972.

2. Smith, R. G., Whang-Peng, J., Gallo, R. C., Levine, P., and Ting, R. C.: Selective Toxicity of Rifamycin Derivatives for Leukaemic Human Leucocytes. Nature New Biology 236: 166-171, 1972.

3. Gillespie, D., Marshall, S., and Gallo, R. C.: RNA of RNA Tumour Viruses Contains Poly A. Nature New Biology 236: 227-231, 1972.

4. Waalkes, T. P., Adamson, R. H., Riddick, D., and Gallo, R. C.: Evaluation of Anti-Tumor Agents on tRNA Methylase Activity of Leukemia L1210. Arch. Int. Pharmacodyn. 196: 345-352, 1972.

5. Robert, M.S., Smith, R. G., Gallo, R. C., Sarin, P. S., and Abrell, J.W.: Viral and Cellular DNA Polymerase: Comparison of Activities with Synthetic and Natural RNA Templates. Science 176: 798-800, 1972.

6. Fujioka, S., O'Hopp, S., Gerber, P., and Gallo, R. C.: Comparison of Chromatographic Patterns of Low Molecular Weight RNA from Burkitt Lymphoma, Infectious Mononucleosis, EB-Virus Transformed, and Normal Human Lymphoblasts. Blood 39: 610-620, 1972.

7. Gallo, R. C., Smith, R. G., Whang-Peng, J., Ting, R. C. Y., Yang, S. S., and Abrell, J. W.: RNA Tumor Viruses, DNA Polymerases, and Oncogenesis: Some Selective Effects of Rifampicin Derivatives. Medicine 51: 159-168, 1972.

8. Gallo, R. C., Abrell, J. W., Robert, M. S., Yang, S. S., and Smith, R.G.: Reverse Trnascriptase from Mason-Pfizer Monkey Tumor Virus, Avian Myeloblastosis Virus, and Rauscher Leukemia and Its Response to Rifampicin Derivatives. J. Nat. Cancer Inst. 48: 1185-1189, 1972.

9. Yang, S. S., Herrera, F., Smith, R. G., Reitz, M., Lancini, G., Ting, R., and Gallo, R.: Rifamycin Antibiotics: Inhibitors of Rauscher (Murine) Leukemia Virus Reverse Transcriptase and of Purified DNA Polymerases from Human Normal and Leukemic Lymphoblasts. J. Nat. Cancer Inst. 49: 7-25, 1972.

10. Schrecker, A. W., Sporn, M. B., and Gallo, R. C.: Inhibition of RNA-Dependent DNA Polymerase by Thymidylate Derivatives. Cancer Res. 32: 1547-1553, 1972.

11. Gallagher, R. E., Ting, R. C., and Gallo, R. C.: A Common Change of Aspartyl-tRNA in Polyoma and SV40 Transformed Cells. Biochim. Biophys. Acta 272: 568-582, 1972.

12. Sarngadharan, M. G., Sarin, P. S., Reitz, M. S., and Gallo, R. C.: Reverse Transcriptase Activity of Human Acute Leukemic Cells: Purification of the Enzyme, Response to AMV 70S RNA, and Characterization of the DNA Product. Nature New Biology 240: 67-72, 1972.

13. Smith, R. G., and Gallo, R. C.: DNA-Dependent DNA Polymerase I and II from Normal Human Blood Lymphocytes. Proc. Nat. Acad. Sci. U.S.A. 69: 2879-2884, 1972.

14. Reitz, M. S., Jr., Abrell, J. W., Trainor, C. D., and Gallo, R. C.: Precipitation of Nucleic Acids with Cetyltrimethylammonium Bromide: A Method for Preparing Viral and Cellular DNA Polymerase Products for Cesium Sulfate Density Gradient Analysis. Biochem. Biophys. Res. Comm. 49: 30-38, 1972.

15. Abrell, J. W., Smith, R. G., Robert, M. S., and Gallo, R. C.: DNA Polymerases from RNA Tumor Viruses and Human Cells: Inhibition by Polyuridylic Acid. Science 177: 1111-1114, 1972.

16. Gallagher, R. E., Walter, C. A., and Gallo, R. C.: Uptake and Aminoacylation of Exogenous Transfer RNA by Mouse Leukemia Cells. Biochem. Biophys. Res. Commun. 49: 782-792, 1972.

17. Bobrow, S. N., Smith, R. G., Reitz, M. S., and Gallo, R. C.: Stimulated Normal Human Lymphocytes Contain a Ribonuclease-Sensitive DNA Polymerase Which is Distinct from Viral RNA-directed DNA Polymerase. Proc. Nat. Acad. Sci. U.S.A. 69: 3228-3232, 1972.

18. Wu, A. M., Ting, R. C., Paran, M., and Gallo, R. C.: Cordycepin Inhibits Induction of Murine Leukovirus Production. Proc. Nat. Acad. Sci. USA 69: 3820-3824, 1972.

19. Gallo, R. C., and Ting, R. C.: Cancer Viruses. In, CRC Critical Reviews in Clinical Laboratory Sciences, December 1972, pp. 403-449.

20. Reitz, M., Gillespie, D., Saxinger, W. C., Robert, M., and Gallo, R.C.: Poly (rA) Tracts of Tumor Virus 70S RNA Are Not Transcribed in Endogenous or Reconstituted Reactions of Viral Reverse Transcriptase, Biochem. Biophys. Res. Comm. 49: 1216-1224, 1972.

21. Gallo, R. C., Hecht, S. M., Whang-Peng, J., and O'Hopp, S.: N^6-(Δ^2-isopentenyl) adenosine: The Regulatory Effects of a Cytokinin and Modified Nucleoside from tRNA on Human Lymphocytes. Biochem. Biophys. Acta, 281: 488-500, 1972.

22. Gillespie, D., Takemoto, K., Robert, M., and Gallo, R. C.: Poly (A) in Visna Virus RNA. Science 179: 1328-1330, 1973.

23. Gallo, R.C.: On the Etiology of Human Acute Leukemia. Medical Clinics of North America 57: 343-354, 1973.

Serial No. NCI-6119
1. Laboratory of Tumor Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cytogenetic Studies: in human neoplastic, hematologic, and congenital diseases; to determine possible correlation between development of leukemia and total body irradiation in long-surviving lymphoma patients; to correlate chromosomal findings in spleen with pathological staging in lymphoma; to determine sex of surviving PHA-responsive lymphocytes in patients receiving blood from donors of opposite sex; to further evaluate chromosomal abnormalities caused by MTX in psoriasis treatment; to observe in vivo and in vitro effects of drugs and viral transformation. Isolation and culture of macrophages. The study of the growth cycle of small lymphocytes and macrophages by means of diffusion chambers in animals.

Previous Serial Number: SAME

Principal Investigator: Jacqueline Whang-Peng

Other Investigators: Seymour Perry, Paul Carbone, Edward S. Henderson, Robert G. Graw, Jr., George Canellos, Harvey Gralnick, Richard Adamson, Ralph Johnson, Elizabeth Chu, Alan Wu, Arnold Ratner, Geraldine Schechter.

Cooperating Units: Clinical Oncology, NCI; Hematology and Supportive Care Branch, NCI; Medicine Branch, Clinical Pathology Department, Clinical Center; Radiation Branch, NCI; Laboratory of Pathology, NCI; Scientific Director, Bionetics Litton Industries; Hematology Research, Veterans Administration Hospital; Dermatologist (private practice), Bethesda, Maryland.

Man Years:

Total: 6
Professional: 4
Other: 2

Project Description:

Objectives:

1. Cytogenetic studies in patients with leukemia or other malignancies; congenital diseases.

2. Cytogenetic studies to determine the possible correlation between development of leukemia and total body irradiation in long-term surviving lymphoma patients.
3. Cytogenetic study of spleen tissue to correlate chromosomal findings in spleen with pathological staging in lymphoma.
4. Chromosomal sex determination of surviving PHA-responsive lymphocytes in patients receiving blood from donors of opposite sex.
5. Further evaluation of chromosomal abnormalities caused by MYX in psoriasis treatment.
6. Observation of in vivo and in vitro effects of drugs and viral transformation on chromosomes.
7. Study of growth cycle of small lymphocytes and macrophages by means of diffuse chambers in animal.
8. Isolation and culture of megakaryocytes.
9. Further evaluation of the cell maturation factor in both liquid and agar system.

Major Findings:

1. Observation of surviving PHA-responsive donor lymphocytes in patients receiving blood from the opposite sex. These cells have been demonstrated to be viable and capable of division as long as one week after transfusion. Spontaneous transformation of donor and host cells was found after 5 days of culture in 5 cases. This phenomenum may be analogous to the in vitro mixed-leucocyte reaction.

2. Methotrexate treatment in psoriasis: low doses (0.002-0.02 ug/ml) of MTX increases the incidence of numerical aberrations in PHA-stimulated lymphocytes in vitro, but produces no inhibition of the mitotic index.

3. The Ph^1 chromosome was noted in at least 5 patients presenting with a diagnosis of lymphosarcomatous cell leukemia.

Honors and Awards

Editorial Board of Blood, 1973.

Publications:

1. Geroge P. Canellos, Jacqueline Whang-Peng, Lowell Schnipper, and Clarence H. Brown III: Prolonged cytogenetic and hematologic remission of blastic transformation in chronic granulocytic leukemia. Cancer 30: 288-293, 1972.

2. Michael M. Hart, Jacqueline Whang-Peng, Susan M. Sieber, Sergio Fabro and Richard H. Adamson: Distribution and Effects of DDT in the Pregnant Rabbit. Xenobiotica 2: (No. 6), 567-574, 1972.

3. R. G. Graw, Jr., R. A. Yankee, G. N. Rogentine, B. G. Leventhal, G. P. Herzig, R. H. Halterman, C. B. Merritt, M. H. McGinniss, G. R. D. Krueger, J. Whang-Peng, R. L. Carolla, D. S. Gullion, M. E. Lippman, H. R. Gralnick, C. W. Berard, P. I. Terasaki, and E. S. Henderson: Bone marrow transplantation from HL-A-matched donors to patients with acute leukemia (Toxicity and Antileukemic Effect). Transplantation 14: (No. 1): 79-90, 1972.

4. J. Whang-Peng, S. Leikin, C. Harris, E. Lee, and J. Sites: The transplacental passage of fetal leukocytes into the maternal blood (36955). Proceedings of the Society for Experimental Biology and Medicine 142: 50-53, 1973.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR MEDICAL ONCOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1972 - June 30, 1973

The Medical Oncology Area has had major responsibility for clinical investigation in new drug development and improvement of therapy of malignant diseases. In the past 20 years, emphasis had been placed on patients with acute leukemia and malignant lymphomas. In the more recent years stress has been shifted to include more activities in the solid tumors (slow growing or low growth fraction tumors) such as lung, ovarian and breast cancers usually limited to treatment of advanced stages. In the past year, major efforts have been made to expand the intramural efforts without adding new resources by increasing coordination with other branches of the National Cancer Institute, particularly Radiation, Immunology, and Surgery so that protocols have been developed to include combined modality therapies of cancers of breast, ovary, melanoma, and pediatric sarcomas. In addition, our senior staff members have assumed roles in directing or working collaboratively with contract supported activities at other institutions both locally and nationwide. These efforts are particularly strong in lung and breast cancer, where our senior staff is involved in the Lung Warking Party and the Breast Cancer Task Force encompassing several institutions, other divisions of the National Cancer Institute, and cooperative groups.

To facilitate the shift in emphasis we have had to make several major program changes. Because the Clinical Center has been severely stressed in the last few years with shortage of supporting personnel, we had to examine our current activities to see which programs should be de-emphasized to start up our new studies. The result was a deliberate attempt to de-emphasize adult acute leukemia and chronic leukemia at the Clinical Center and shifting resources and efforts to pediatric tumors, breast cancer, and combined chemotherapy and immunotherapy. Thus the Hematology and Supportive Care Branch was changed to the Pediatric Oncology Branch, with three sections - Chemoimmunotherapy, Experimental Hematology, and Infectious Diseases. These sections will emphasize cure-oriented treatment trials using combined modalities and intensive supportive care in patients with childhood lymphomas, neuroblastoma, rhabdomyosarcoma, and childhood acute leukemia. Dr. John L. Ziegler, formerly Director of the Uganda Cancer Institute was named Branch Chief in FY 1973.

In the Office of the Associate Director, a Medical Breast Cancer Service under Douglass C. Tormey, M.D., Ph.D., was established, assigned beds, clini-

cal associates and staff, to develop a program with the Surgery Branch, in breast cancer. Dr. Tormey also assists the Associate Director, who is Chairman of the Treatment Committee of the Breast Cancer Task Force, in managing a contract program involving developmental and applied biologic studies as well as treatment with surgery, chemotherapy, hormonal therapy and radiation therapy. Closely integrated trials have been set up with area governmental hospitals using agreed protocols.

Sections were also established in the Medicine Branch, affording recognition to our staff efforts in Cell Kinetics, Clinical Pharmacology, and Hematology Studies. The NCI-VA Medical Oncology Branch administratively located at the Washington Veterans Administration Hospital using VA personnel, also has established assistant chiefs for pharmacology, clinical activities, pulmonary pathology and education-supportive care. During FY 1973 an international meeting on lung cancer was held at Airlie House and contracts were established to develop major collaborative multidisciplinary efforts at several institutions.

During this past year through the efforts of an Education Committee consisting of representatives from each of the clinical branches and services, an integrated training program was developed to allow our junior staff, while they are assigned to the National Cancer Institute, an opportunity to get a broad experience in medical oncology and to satisfy requirements of the new subspecialty certification of Medical Oncology. In addition to formalizing the 2 year experience of our junior staff, since the doctor draft has expired, the program as defined should continue to attract outstanding young men to participate in clinical care and research. The senior staff feels strongly that with continued support of positions, we should attract these young men who have been the backbone of our clinical programs.

The Uganda Cancer Institute has had a major change in personnel and goals in FY 1973. Because of the internal political problems in Uganda, our three NCI staff members have had to leave the contry and the clinical scope of activities contracted. Fortunately, Dr. Ian Magrath, a British physician assigned to Makerere and our unit, was able to continue the Lymphoma Treatment Center until Dr. Charles Olweny was able to complete his training and return to Uganda in March 1973. Dr. John Ziegler will plan to make an on site visit as soon as practical, to develop future program plans.

Program Accomplishments

The members of the Medical Oncology Area continue to be highly productive and recipients of national recognition. Over 200 papers were published or are in print from work done during FY 1973. Our staff spends considerable time giving lectures and presenting papers at local, national and international meetings. The following items are intended only to highlight certain important accomplishments. The details can be obtained in the individual project reports.

Preclinical

DNA Viral Studies - IXX

Dr. Arthur Levine (Section on Infectious Diseases, Pediatric Oncology Branch, NCI-6830) and his laboratory staff have characterized several AD2-SV40 hybrid viruses that can be used to study the sequence of genes in the SV40 virus and to study malignant transformation. These hybrids have permitted the mapping of certain genes in the SV40 virus which may be responsible for oncogenic potential of this virus.

Marrow Culture Studies - IXX

An important finding has been the demonstration that human marrow cells grown in semi-solid media can be induced to form colonies in vitro by addition of colony stimulating factor (NCI-3423, 3405). In the laboratory we have demonstrated that certain leukemic blasts can be made to differentiate in vitro. The possibility of finding a physiologic factor capable of causing abnormal cells to produce granulocytes or stimulate marrow cells to produce myeloid elements offers a new approach to controlling the diseases of acute leukemia and marrow failure. Studies have indicated that leukemic cells inhibit the growth of normal marrow elements by immunologic cell killing mechanisms of normal marrow cells and neoantigens (NCI-3423).

New Drug Development - 2AX

Streptozotocin (NCI-6500) is unique because of its demonstrated lack of marrow toxicity. Widespread clinical use has been hampered by its propensity to induce renal damage in man and diabetes in animals. Studies by Dr. Philip Schein (Section on Clinical Pharmacology, Medicine Branch) of 3-(tetra-acetyl glucopyranos-2-yl)-1-2-chlorethyl (GCNU) offer this drug as a promising candidate because it has activity against L1210 but no marrow toxicity. Further analogues are available for comparative studies.

Cell Cycle Kinetic Studies - 4XX

The understanding of cell cycle parameters of tumor and pharmacokinetics should allow for improved therapeutic results (NCI-3420). Efforts to understand in experimental systems the pharmacokinetics of methotrexate, cyclophosphamide, nitrosoureas as related to changes in cell cycle characteristics has been a major part of the Section on Cell Kinetics, Medicine Branch. A major advance in technology seems to be possible by following the changes in DNA H^3TdR specific activity in both the tumor and normal tissues. Studies to date indicate by evaluating the changes in H^3TdR specific activity rapid analysis can be done indicating a variation in the rate of recovery by normal versus tumor tissue. The tumor tends to recover more slowly suggesting that retreatment times can be estimated to maximize tumor cell kill and sparing normal tissues.

A major study of cell kinetics in Burkitt's tumor was published this year

(see NCI-6800) Iverson et al.). The findings indicated GF at 100% with cell cycle times of 20-30 hours.

Phase I Studies - 5XX (NCI-3403, 3424)

During the past year the following drugs were in Phase I trials: Isophosphamide (NSC 109724), (±)1,2-di(3,5-dioxopiperazin-1-yl) propane (NSC 129943), epipodophylotoxin ethylidene glucoside (NSC 141540), thalicarpine (NSC 68075), methyl CCNU (NSC 95441), and procarbazine (NSC 77213). In keeping with the program concept of doing pharmacology studies as early as possible - most of these drugs were evaluated for their pharmacologic disposition as well.

Phase III Studies - 6X1

A. Acute Leukemia

Major efforts of combined chemotherapy and immunotherapy have now reached the point where early results are available (see NCI 6800, 6810). In protocol #72-1, children with ALL were induced with POMP, underwent CNS prophylaxis with radiation and consolidation chemotherapy and then to maintenance chemotherapy with MTX alone or with BCG and cells. To date no differences in relapse rates are seen between chemotherapy (2 arms) 3/21 or immunochemotherapy 2/12). A similar observation was seen for a second protocol (#72-2) for previously treated patients. Despite some evidence for enhanced immunity with BCG plus cells, there was no difference in remission duration.

B. Burkitt's Tumor (NCI-6800)

Over 162 patients have now been treated at the Lymphoma Treatment Center. Survival is related to Stage (82% 4 years NED for I and II). The overall predicted 200 week survival is 57%. Emphasis in the program has been on use of BCG immunotherapy and CNS treatment. As in patients with acute leukemia, BCG administration to patients in remission with cytoxan offered no advantages as compared to no treatment. Relapses occurred in 9/17 and 10/16 patients respectively. In the study of CNS disease treatment CCNU at 70 mg/m^2 had no effect on preventing CNS recurrences. Currently a study attempting to evaluate Ara-C plus MTX with or without HU is being carried out.

Updating the experience with American Burkitt's tumor (NCI-3403) indicates that 11 of 29 patients are alive and free of disease 3+ years after cytoxan. The longest is now 8 years post treatment.

Hodgkin's Disease (NCI-3403)

The major findings this fiscal year include the observation that the MOPP regimen continues to induce 75% or more of patients with Stage IV Hodgkin's and 50% long-term disease-free remissions. However, in a randomized study to evaluate the optimal duration of treatment the results to date indicate that

continued MOPP or BCNU beyond 6 or 8 months fails to add to disease-free interval - but does increase toxicity. A second important finding has been a surprisingly high proportion of second tumors in patients treated with both MOPP and total nodal radiotherapy. These tumors include sarcomas, epidermoid cancers, and leukemias. Procarbazine, a component of the MOPP therapy, has been shown to be highly carcinogenic - particularly when combined with alkylating agents or radiotherapy.

Lung Cancer (NCI-3024)

The recognition that cell type plays an important role in survival was confirmed by a randomized study comparing 2 and 3 drug combinations. Despite similar treatment schedules only small cell carcinoma was significantly affected by 3 drug treatment. Utilizing the Lung Working Party, further studies of small cell carcinoma will include a study of prophylactic radiotherapy to the CNS and upper abdomen - known sites of metastases. In the Lung Working Party specific protocols have been developed for each of the major cell types.

Breast Cancer - 6X1 (NCI-3403, 6700)

The results of combination chemotherapy offers the conceptual advance needed to begin to develop curative therapy programs. In a pilot program on the Medicine Branch (Dr. Canellos) utilizing FU, cytoxan, MTX, and prednisone, 16 of 24 patients had clinically useful responses. The median duration is 6+ months (range 2-18 months).

The Medical Breast Cancer Service assumed its clinical beds in January 1973. Major emphasis has been placed on use of combined surgery plus chemotherapy protocols, hormonal manipulation plus chemotherapy and combination chemotherapy. Adriamycin has recently been shown to be the most effective single agent in breast cancer producing results comparable to some combinations. The Medical Breast Cancer Service protocols in advanced disease are designed to incorporate this new agent into combination chemotherapy protocols utilizing adriamycin in combination with cytoxan and FU; other institutions are examining ADR + vincristine or ADR + methotrexate. Besides the chemotherapy efforts, studies are underway to study tumor cell markers, radionuclide scanning procedures, and predictive estimators of high risk recurrence. Collaborative efforts include the contract efforts with the Mayo Clinic, Memorial Hospital, Albany Medical Center, Roswell Park Memorial Institute, U.S. Naval Medical Center, Bethesda, Walter Reed Army Medical Center, Washington, D. C.; the Breast Cancer Task Force, ECOG, and ALGB, and Milan Cancer Institute, Milan, Italy, NSABP. Data collection and reference center has been established to keep abreast of the various studies and provide information on NCI contract results.

Ovarian Cancer - 4X1 (NCI-3403)

A major killer of women in the USA, ovarian cancer has been shown to be responsive to several chemotherapeutic agents. Preclinical attempts at developing an effective combination therapy have been started, both in-house and on

contract with M. D. Anderson Hospital for Phase II studies. These studies are combined with other programs designed to evaluate cell kinetics and tumor cell markers (NCI-3404).

Non-Hodgkin's Lymphoma - 4X1 (NCI-3403)

Advances in the treatment of non-Hodgkin's lymphoma have resulted from the recognition of the importance of histologic subtypes and the use of combined chemotherapy programs with radiotherapy. In collaboration with Radiotherapy Branch, major protocols have been developed to treat all stages with randomized as well as combined modalities in some stages. The results indicate that combined therapy produces a high remission rate, recurrence occurs more frequently than in Hodgkin's, but long-term disease-free survival can be seen in about 30% of patients.

Other Tumors

Major collaborative efforts have been established between our Pediatric and Medicine Branches, and other NCI departments, to study melanoma (NCI-3403) and osteogenic sarcoma patients (NCI-3403, 6803).

The studies in hepatocellular carcinoma (NCI-6800) have indicated that several markers are present that can be used to evaluate disease extent. These include alpha fetoprotein (AFP), proline hydroxylase, chorionic gonadotropin (HCG), and abnormal fibrinogens. Despite the development of a clinically useful staging scheme, no clinically useful chemotherapy scheme has been developed using single or combined agents.

Kaposi's sarcoma (NCI-3403) has yielded with improved therapeutic results employing combined Actinomycin D plus vincristine. Currently, DTIC, another effective agent, will be incorporated into the treatment protocol.

Malignant melanoma in Africa seems to be much more resistant disease to chemotherapy (NCI-6800). Earlier studies with immunotherapy for post-op stage II patients delayed recurrences. Further studies are being planned.

Laminar Air Flow Project - 6X1 (NCI-6830)

A major study was completed this year confirming in a controlled protocol the value of the protected environment in controlling the infectious complications of patients with acute lymphocytic and myelocytic leukemia undergoing induction therapy. Since our program emphasis has shifted away from this patient population, efforts will be made to include other solid tumors in the units, such as ovarian histiocytic lymphomas, and some of the pediatric tumors.

The severe shortage in Clinical Center personnel will continue to affect our programs, particularly in our attempt to establish a Protective Environment-Intensive Care Ward on 2-B. FY 1973 did provide for construction of facilities but development of staffing still must be worked out. Hopefully, through changes in the Clinical Center staffing and additional contract sup-

port we will be able to provide a highly effective advance system type facility not only to continue research efforts but also to provide organization of manpower and procedures so that the National Cancer Institute and other institutions will be able to have solid data on these highly specialized facilities.

Blood Component Transfusion Therapy - 6X1 (NCI-6820)

Support therapy, particularly non-red cells for patients with malignancies represents a major research effort since hemorrhage and infection are the two major causes of death. The pioneering work on platelet and white cell transfusions were done at the Clinical Center. In the past year a major breakthrough has occurred in our methodology to provide long-term support. Transfusion therapy in the past was done by massive infusions of multiple random donors. Even in patients receiving immunosuppressive therapy resistance was seen within a few months. Recognizing that the HL-A system of lymphocyte typing might serve as a marker for white cell and platelet transfusion compatibility, a tissue typing contract was established with UCLA under Dr. Paul I. Terasaki linked by computer to the NCI and by mail for specimens.

A firm relationship has been established not only for compatible siblings but also for random and HL-A matched donors. This has allowed the utilization of selected specific donors where multiple random donor resistance is present. However, there is now evidence accumulating that additional platelet and granulocyte antigens are seen that may need to be taken into account.

Not only are the improvements in typing being made but also in procurement. These have included modifications of the NCI-IBM Cell Separator bowl or extracorporeal collecting systems. To evaluate these and for other studies, dog models for WBC transfusions have been developed.

Bone marrow ablation and transplantation offers a simplistic approach to cure a marrow disease either malignant or benign (aplastic anemia, Sickle cell disease, etc.). The major obstacle to success has been the development of graft-versus-host disease because not only are marrow stem cells transfused but also immunocompetent lymphocytes. In both preclinical and clinical studies, efforts are underway to determine optimal methods to ablate all host marrow for acceptance of donor marrow with minimization of GVH. Clinical trials in the past year with marrow transplantation have included 3 patients with far-advanced ALL who received large amounts of cyclophosphamide, aracytosine, thioguanine, and BCNU. Two patients died within 30 days and one lived 5 months. In contrast, a patient with CML failed to have his Ph^1 positive marrow eradicated even with these high doses. Two patients with identical twins without leukemia were treated with this combination. Both leukemic patients have had successful remissions and takes. Two additional patients with far-advanced refractory leukemia received autologous marrow without evidence of successful takes following the intensive combination. To date, one patient treated with immune suppression and tumor cell ablation has survived 18 months with no evidence of disease. A major review of this program area will be done in FY 1974 to determine its position in our

changing program.

A major program change will take place in FY 1974. Because of the marked change in donors from random single unit blood bank by purchase, to specific, multi-unit donors with HL-A matching, we have had to rely on our limited resources to provide for plateletpheresis and white cell separations. The Clinical Center and its Blood Bank have agreed to cosponsor a contract to provide on site or close-by donor collection facilities for platelets that should free up our own personnel and space for more research programs.

Honors and Awards

Dr. Paul P. Carbone

1. Invited by Dr. John Beck, Chairman, American Board of Internal Medicine, to serve on the Oncology Test Committee of the Board to develop examinations for subspecialty certification in oncology.

2. Invited to moderate the New York State Programs Association, Inc. (Nov. 1972) annual meeting and to present a lecture entitled "The Cell Cycle and Practical Considerations in Chemotherapeutic Treatment of Breast Cancer".

3. Invited by Dr. K. D. Berlin, Regents Professor and President OSU Sigma Xi Chapter, to speak on recent breakthroughs in the treatment of cancer at the Sigma Xi Spring Banquet, April 18, 1973.

4. Invited by Dr. W. G. Sauer to present lecture at Mayo Clinic, on "Malignant Lymphomas: Recent Advances in the Natural History and Chemotherapy".

5. Invited to present an American Cancer Society Lecture on "New Concepts of Patients with Lymphoma" at the University of Pittsburgh School of Medicine, on May 15, 1973.

6. Invited to serve on Board of Governors of the American Federation of Clinical Oncology Societies, July 1, 1972 through June 30, 1973.

7. Albert and Mary Lasker Foundation Medical Research Award, November 1972, for Clinical Cancer Chemotherapy.

8. Member of Scientific Delegation to the USSR, November 1972.

9. Invited Lecturer at Georgetown University Medical School, Feb. 1973.

10. Invited by Dr. John A. Richman to present a lecture before the members of the Scientific Research Society of America, June 1973.

11. Appointed Chairman of the Chemotherapy Research Committee, NCI.

12. Appointed Deputy Clinical Director, NCI in 1972.

13. Invited to participate in the Conference on Chemotherapy, Rio de Janeiro, Brazil, July 1972, and to present paper entitled "The Role of Chemotherapy in the Treatment of Hodgkin's Disease".

14. Invited to present a lecture on "Chemotherapy of Lymphomas" at the XIV International Congress of Hematology, Sao Paulo, Brazil, 1972.

15. Invited to present a talk on chemotherapeutic treatment of cancer, by Dr. Jose Lozano Pardo, Lima, Peru, July 1972.

Honors and Awards (continued)

Dr. Paul P. Carbone

16. Member of the Organizing Committee, Second International Workshop on Hemopoiesis in Culture, Airlie House, Warrenton, Virginia, May 23-26, 1973.

17. Invited to serve on the American Society of Hematology's Subcommittee on Cell Proliferation.

Publications

1. Carbone, P. P.: Non-Hodgkin's lymphoma: Recent observations on natural history and intensive treatment. Cancer 30: 1511-1516 (Dec.) 1972.

2. Carbone, P. P.: Report on Workshop: Chemotherapy, presented at the International Symposium on Hodgkin's Disease (Stanford University School of Medicine, March 20, 1972) - NCI Monograph 36, 1973.

3. Kennedy, B. J., Calabresi, P., Carbone, P. P., Frei, E., III, Holland, J. F., Owens, A. H., Jr., Sleisenger, M. H., and Beck, J. C.: Training Program in Medical Oncology. Ann. Intern. Med. 78: 127-130 (Jan.) 1973.

4. Carbone, P. P.: Management of patients with hon-Hodgkin's lymphoma. Arch. Intern. Med. 131: 455-459 (March) 1973.

Serial No. NCI-3423
1. Medical Oncology
2. Office of the Associate Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: In Vitro Marrow Granulocyte Culture

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone, M.D., Joan M. Bull, M.D. Robert W. Sponzo, M.D. and Alan Rosenblum, M.D.

Other Investigators: Eleanor Stashick, Wilmarie McKoy, J. Whang-Peng, M.D. P. Carmeci, and T. Anderson, M.D.

Cooperating Units: Medicine Branch, MO,DCT,NCI; Pediatric Oncology Branch, MO, DCT,NCI, Laboratory of Tumor Cell Biology, ET,DCT, NCI; Department of Technical Development, NHLI

Man Years:

Total: 6
Professional: 3.2
Other: 2

Project Description: (1XX)

Objectives:

1. Studies of In Vitro Granulocyte Production of Patients with Hodgkin's Lymphoma.

To assess the acute and chronic effects of tumor and chemotherapy on bone marrow function, the in vitro granulocyte colony formation (CFU-c) was studied in patients with advanced lymphoma before and after treatment. Using a semi-solid methylcellulose system the CFU-c of 42 normal volunteers was 2.98 (0.9 to 6.25). The mean CFU-c at diagnosis of 19 patients with advanced Hodgkin's disease was low, 1.22 (0.00 to 2.4), differing from control values ($p < .005$). The CFU-c of patients with documented marrow involvement did not differ from advanced stage patients with proven marrow disease. In non-Hodgkin's lymphoma, patients with diffuse histiocytic and lymphocytic histologies had lower CFU-c's than those with nodular pattern. The mean CFU-c of 15 patients with diffuse lymphoma was 1.04 (0.00 to 2.00), and differed from controls ($p < .005$), whereas the mean CFU-c of 15 patients with nodular lymphoma did not differ from controls (mean 2.15, range 0.38

to 6.10). The cellular composition of the tumor (histiocytic, lymphocytic, or mixed) did not relate to CFU-c. Two patients studied serially prior to each of 6 cycles of MOPP therapy had low initial CFU-c's. CFU-c'c entered the normal range following the first cycle of chemotherapy and remained normal during subsequent courses. Three of 4 patients in remission and off MOPP therapy for up to 6 years had normal CFU-c's. Patients with advanced Hodgkin's disease and diffuse histiocytic and lymphocytic lymphoma have impaired CFU-c's prior to treatment, whereas those with nodular lymphomas have normal CFU-c's. The low CFU-c may reflect non-specific inhibitory effect related to the biologic aggressiveness or volume of tumor. The low colony formation returns to and remains normal following successful drug control of the tumor.

2. Altered In Vitro Granulopoiesis in the Presence of Leukemic and Normal Marrow Cells.

Patients with acute leukemia and preleukemia manifest deficient hematopoiesis in vivo and depressed granulopoiesis in vitro although the bone marrow may show only minimal infiltration with abnormal cells. Colony formation is not linearly related to the proportion of normal and abnormal cells in the marrow of these patients and normal colony formation in vitro occurs regularly only when the proportion of blasts is under 10%. Using the in vitro assay for granulocyte colony formation (CFU-c) we examined the response of normal marrow to the addition of small numbers of leukemic cells and other normal marrow cells. Human marrow was cultured in semi-solid methylcellulose, each plate containing 1.0 to 2.0 x 10^5 normal test (recipient) cells to which 1.0 x 10^3 to 1.0 x 10^4 unrelated (donor) cells were mixed prior to plating. Results were expressed as a percentage change from the sum of control plates.

Leukemic cells in concentrations of 0.5 to 5.0% uniformly inhibited growth of normal marrow cells to 48% of control levels (range 32-63%, 10 values from 3 patients). Addition of normal marrow cells to a normal recipient marrow produced marked stimulation to 255% of control (range 210-290%, 10 values from 6 normals) if the donor/recipient pair was HL-A identical or resulted in inhibition to 48% of control (range 45-56%, 11 values from 5 normals) if the donor/recipient pair was mismatched for 3/3 or 4/4 antigens. Mismatches for 2/4 or 3/4 antigens resulted in intermediate responses. These patterns were reproduced using irradiated or freeze-thawed donor cells as well as supernates from freeze-thawed cells.

These results demonstrate that small proportions of leukemic as well as normal unrelated marrow cells may alter granulopoiesis in vitro. The interesting relationship to HL-A compatibility suggests one mechanism of inhibition by leukemic cells in vivo may involve recognition and response to neo-antigen.

3. Capillary Tube Scanning Applied to In Vitro Mouse Marrow Granulocyte Growth.

A technique was utilized for electronic monitoring and counting of mouse marrow granulocyte colonies in capillary tubes. Capillary tube scanning allowed daily monitoring of replicate samples, and provided kinetic data not shown by examination of standard semisolid cultures in petri dishes. The method shows growth as early as day 1 that is consistent in pattern and sensitive to dose inoculated. Cloning particles greater than 20 μ could be detected; because of this, counting efficiencies were 10-100 times higher than were cloning efficiencies in conventional petri dishes. The technique demonstrated growth as early as day 1 that was consistent in pattern to dose of cells inoculated.

4. The Effect of Androgens on Human CFU-c.

In vitro culture of bone marrow from normal individuals has been used to assess the effects of androgens on hematopoietic tissue. Colony forming ability for normal individuals was standardized using marrow from 14 normal males (4.4 col/10^4 cells plated) and from 8 normal females (3.0 col/10^4 cells plated). In addition to this difference in absolute colony forming ability, the dose-response curves for increasing cell concentrations varies significantly between males and females, suggesting that females have a decreased plating efficiency. Testosterone propionate was added to cultures in increasing concentrations to establish a dose-response relationship. At very low concentration, 3 x 10^{-12}M, no effects were seen. At concentrations from 3 x 10^{-10} to 3 x 10^{-8}M, increasing stimulation of colony forming ability was noted, reaching a maximal level of 35% above controls. At levels higher than 10^{-7}M inhibition of colony formation was seen to approximately 35% of normal. These findings suggest that androgens may be both stimulatory and inhibitory to marrow growth in vitro, and the implications for their use in vivo will be investigated further.

5. Studies are currently underway to compare the in vitro methyl cellulose bone marrow culture technique with other methods for growing human bone marrow cells. A liquid culture system has been used with McCoy's standard solution as the culture medium. Growth of cells over a 3 week interval has been observed, with stimulated cultures growing better than unstimulated. Granulocytic differentiation has been observed in this system. A methyl cellulose culture system in tubes has also been evaluated. Growth of cells, colony formation, and granulocytic differentiation have all been observed. Stimulated cells have a greater growth efficiency than unstimulated and this data correlates well with the observed growth of marrow cells on plates. In two instances the growth of AML marrow cells in tubes yielded good colony formation, while on plates few colonies were seen.

An improved method of counting granulocytic colonies is also being developed. Using a capillary technique to assay for aggregates of cells (small colonies), the growth of colonies in tubes has been studied. The growth of colonies can be detected and the growth efficiency of these cells can be determined by this technique. Data to date suggest that this method correlates well with macroscopic colony counting, giving similar estimates of growth efficiency. This

method also Has the advantage of early detection and evaluation of colony growth before visible colonies are present.

Separation studies of human bone marrow cells have also been performed, using a 1G sucrose gradient technique. These studies are intended to yield relatively pure samples of specific human bone marrow cell types. These samples will then be placed in our in vitro marrow culture system and the growth potential of these cellular fractions as well as the cell- cell interaction will be studied.

6. The second International Workshop on Hemopoiesis in Culture will be held at Airlie House, May 23-26, 1973, sponsored by the National Cancer Institute. Members of the laboratory of the Associate Director for Medical Oncology have played a major role in developing the agenda and organizing the meeting. Seventy five individuals from around the world will participate. A publication of the proceedings is planned. Work in this area has potential significant influence in understanding not only the physiology of human marrow but also understanding the leukemogenesis. Another direct effect will be the development of large quantities of purified human conditioning factor on contract for use in man to study the possible differentiation of leukemic cells as a method of controlling the disease.

Future Course of Project:

1. Additional cytogenetic studies of marrow from patients with acute, chronic and pre-leukemia will be continued to identify those patients whose leukemic cell lines show evidence of maturation with colony stimulating factor.

2. Drug toxicity studies with an investigational drug (D-114460) (GCNU) that does not effect peripheral granulocyte counts, will be compared with bis-dichloronitrosourea (BCNU) in the murine system to determine the drug effects on CFU-c.

3. The determination of optimum dose scheduling of cytoxan and adriamycin to minimize toxicity to CFU-c will be investigated in the murine system and in selected patient studies.

4. The effect of androgens, estrogens, and progesterones on normal in vitro granulopoiesis will be studied in patients with primary and metastatic mammary cancer.

5. The potential application of human granulocyte stimulating factor to cause differentiation of leukemic cells as a method of disease control will be pursued. This material may also be of great significance in stimulating granulopoiesis.

Honors and Awards:

Dr. Joan M. Bull

1. Presented a paper on studies of in vitro granulocyte production of patients with Hodgkin's lymphoma, at the American Society of Hematology meetings, Miami, Florida, December 1972.

2. Invited to present a paper entitled "In vitro colony formation in acute leukemia and lymphoma" at Georgetown University, Division of Hematology, January 1973.

Dr. Robert Sponzo

1. Presented a paper on pharmacology of the nitrosoureas at the Albany Medical Center, Albany, New York, in March 1973.

Dr. Alan Rosenblum

1. Presented paper entitled "Altered in vitro granulopoiesis in the presence of leukemic and normal marrow cells" at Atlantic City, N. J., American Federation for Clinical Research meetings, April 1973.

Publications

1. Bull, J., Duttera, M., Northup, J., Stashick, E., and Carbone, P. P.: Serial studies of in vitro colony formation in patients with acute myelocytic leukemia. Blood (in press).

2. Duttera, M., Bull, J., Northup,J., Stashick, E., and Carbone, P. P.: Serial studies of in vitro colony formation in patients with acute lymphocytic leukemia. Blood (in press).

3. Abrams, L., Carmeci, P., Bull, J. M., and Carbone, P. P.: Capillary tube scanning applied to in vitro mouse marrow granulocyte growth. J. Nat. Cancer Inst. 50: 267-270, 1973.

4. Frieze, T., Lindberge, J., and Bull, J.: Dermatomyositis in the carcinoid syndrome. Arch. Int. Med. 131: #4, April 1973.

5. Arseneau, J., Sponzo, R., Levin, P., Schnipper, L., Bonner, H., Young, R., Canellos, G. P., Johnson, R. J., and DeVita, V. T.: Nonlymphomatous malignant tumors complicating Hodgkin's disease. New Eng. J. Med. 287: 119-1122, 1973.

Serial No. NCI-6308
1. Medical Oncology
2. Office of the Associate Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biostatistical Information Systems Unit

Previous Serial Number: Same

Principal Investigator: Richard Simon, Ph.D.

Other Investigator: Lorraine G. Fitzsimmons, B.A.

Cooperating Units: Medicine Branch, MO, DCT, NCI
Medical Breast Cancer Service, MO, DCT, NCI
NCI-VA Medical Oncology Branch, MO, DCT, NCI
Pediatric Oncology Branch, MO, DCT, NCI

Man Years:

Total: 1.5
Professional: 1
Other: 0.5

Project Description:

The Biostatistical Information Systems Unit was established during FY 1972 in order to utilize statistical methodology and computer technology to maximize knowledge obtained from clinical trials and other research carried out within the Medical Oncology Area of the Division of Cancer Treatment. In addition to direct participation in the design and analysis of clinical trials and other experiments, the Unit conducts research in statistical methodology in support of the above goals. A list of projects in which the Unit is actively involved follows.

1. Participation in the design and analysis of clinical trials. Development of disease and protocol specific evaluation forms for the analysis of clinical trials. Abstraction and computerization of data concerning patient pretreatment characteristics, treatment administered, response, complications, and follow-up information. Development of computer systems and production of reports to assist in the monitoring of clinical trials. Thorough analysis of trial results in disseminated bronchogenic carcinoma, lymphoma, ovarian carcinoma, acute and chronic leukemia, and carcinoma of the breast.

2. Statistical consultation in the design and analysis of retrospective clinical studies. Retrieval of data from computer files for these studies.

3. Abstraction, computerization, and analysis of data concerning the efficiency of procurement and effectiveness of infusion of granulocytes and platelets.

4. Statistical consultation in the design and analysis of laboratory research.

5. Systems analyses of the reporting needs in the clinical environment and development of proposals for the utilization of computer technology to meet these needs with a minimum of duplication of effort.

6. Participation in the design and analysis of Phase II clinical trials performed on contract. Development of a uniform computerized data reporting and analysis system to be used for Phase II clinical trials of carcinoma of the breast performed on contract to the Division of Cancer Treatment. Data will be updated monthly and analyzed centrally. Analysis will consist of evaluation of response and toxicity to chemotherapy, prognostic value of proposed discriminants, impact of study design upon survival, cross-resistance evaluations, etc.

7. Development and implementation of a computerized file of all world-wide clinical trials in carcinoma of the breast supported by the National Cancer Institute. The file includes a description of the patient selection, treatment programs, stratifications used, date the trial began, ended, last reported, and a summary of the results. This file is maintained in collaboration with the Medical Breast Cancer Service.

8. Interfacing between the investigators of the Medical Oncology Area and the computer systems and personnel of the Clinical Center and Division of Computer Research and Technology in order to utilize computerized data available and to eliminate duplication of effort. Obtain retrievals from centralized computer files and develop computer programs to utilize the centralized files for our purposes. Make known to clinical investigators data available, and make known to Clinical Center personnel our priorities for the development of future centralized computer files.

9. Development of stratified randomization schemes and performance of randomizations for clinical trials.

10. Development and application of methodology for the thorough analysis of cell kinetics experiments in human and animal systems. Computer programs developed are being utilized in research institutions throughout the world.

11. Maintenance and report generation for computerized serum bank file.

12. Development of a computerized system for the storage of detailed data concerning the morphology of hematopoietic cells of adults with acute leukemia and the analysis of this data to determine discriminants that predict response to chemotherapy and survival.

13. Statistical Research:

    a. Investigation of sequential termination and treatment assignment rules for the development of clinical trials which require fewer patients and allocate fewer patients to the inferior treatment than is accomplished by traditional formulations.

    b. Development and evaluation of new methods of allocating treatments to patients for clinical trials with few patients and many stratification variables. Traditional methods may result in considerable imbalance of stratification variables for such situations.

    c. Development of mathematical models for relating tumor burden to concentrations of urinary polyamines.

    d. Development of methodology for allocating a panel of platelet donors to thrombocytopenic patients based upon transfusion history, cytotoxic antibody screening, and HL-A typing.

    e. Development of methodology for estimating required sample sizes for clinical trials with non-standard response distributions.

    f. Investigation of multistage designs for Phase II clinical trials for the introduction of new drugs into treatment combinations.

Serial No. NCI-6700
1. Medical Breast Unit
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical Program in Breast Carcinoma

Previous Serial Number: None

Principal Investigators: Douglass C. Tormey, M.D.,Ph.D., Joan M. Bull, M.D. and Paul P. Carbone, M.D.

Other Investigators: R. Sponzo, M.D., L. E. Schnipper, M.D., G. W. Geelhoed, M.D., A. S. Ketcham, M.D., J. Blom, M.D., R. Moquin, M.D., E. Perlin, M.D., T. Pomeroy, M.D., G. O'Conor, M.D., R. J. Margolis, M.D., R. Simon, Ph.D., S. Carter, M.D., J. Whang-Peng, M.D., and T. P. Waalkes, M.D.,Ph.D.

Cooperating Units: Surgery Branch, NCI, Radiation Branch, NCI, Pathology Branch, NCI, Office of the A.S.D. for MO, NCI, Cancer Therapy Evaluation Branch, NCI, Experimental Therapeutics, NCI, Walter Reed Army Hospital, and National Naval Hospital

Man Years:

Total: 3.65
Professional: 2.55
Other: 1.1

Project Description:

The clinical program in breast canrcinoma by the Medical Breast Unit involves three major areas of investigation:

I. Clinical Trials in Breast Carcinoma Patients
II. Ancillary Studies in Breast Carcinoma Patients
III. Extramural Activities in Breast Carcinoma Patients.

The Medical Breast Unit was established in July 1972 under Douglass C. Tormey. It is directly responsible to the Office of the Associate Scientific Director for Medical Oncology, Dr. P. P. Carbone. A secretary joined the program in August 1972. The clinical program draws direct personnel support from the OASD in order to support the clinical program. An interdisciplinary approach to treatment was established during the fall of 1972 with the Surgery Branch, Radiation Branch, and Pathology Branch, NCI. Four beds on 2-East ward were allocated to the Unit on January 1, 1973. As of February 22,

1973, seven patients had been admitted. Clinic space was allocated to 1-1/2 afternoons per week on January 1, 1973; 66 clinic visits were recorded to February 22, 1973. A Clinical Associate was assigned to the program beginning January 1, 1973. The activities in each of the three major areas are outlined below.

I. Clinical Trials

A. Initial eradicative trials in newly diagnosed patients. 7XX

1. The Unit is working with the Surgery and Radiation Branches to arrive at the appropriate role of surgery and radiation in the patient presenting with Stage I, II, or III disease. This is not yet settled.

2. Following primary therapy patients are to be entered into long-term adjuvant chemotherapy programs if they have pathologically positive axillary nodes. At present we are participating in the joint National Surgical Adjuvant Breast Program - Eastern Cooperative Oncology Group investigation which randomizes these patients to receive intermittent therapy with either L-phenylalanine mustard or a placebo. The study was activated in October 1972. The Medical Breast Unit also participates in the Acute Leukemia Group B. It is expected that an adjuvant chemotherapy protocol chaired by Douglass C. Tormey will be activated in the spring of 1973. Therapy in this protocol will be more aggressive than that mentioned above. We plan on participating.

B. Hormone plus Chemotherapy Trials in Patients with a First Recurrence of Disease. These studies are designed to evaluate the role of chemotherapy as an adjuvant to standard hormone therapy in patients presenting with their first recurrence of disease since mastectomy. These programs were designed with the cooperation of the Surgery and Radiation Branches. 7XX

1. Between 5 and 15% of Stage I and II patients present with their first recurrence in the mastectomy site. These patients undergo an excisional biopsy to confirm the recurrence, and are then evaluated clinically to ascertain if there are metastatic foci; if there are they become candidates for studies listed below (2 and 3). If there is no clinical evidence of other disease the patients will enter a protocol now being consumated by the Medical and Surgical Breast Unit programs. The study entails a bilateral oophorectomy and randomization to adjuvant chemotherapy versus observation. This study is expected to be activated in March 1973.

2. Premenopausal patients with a measurable first recurrence will enter a protocol in which they undergo a bilateral oophorectomy

followed by an observation period of 4-12 weeks. If progressive disease occurs in this interval they enter the Second Recurrence program (See C). If a complete response occurs at 8-12 weeks, or a partial response at 12 weeks, they are then randomized to receive either combination chemotherapy or no further therapy. This latter group will receive the cytotoxic regimen when progressive disease develops. This study is presently en route to the Clinical Research Committee.

3. Postmenopausal patients with a measurable first recurrence will enter a protocol in which they will initially receive diethylstilbesterol for 4-12 weeks. If progressive disease occurs in this interval they enter the Second Recurrence program (See C). If a complete response occurs at 8-12 weeks, or a partial response at 12 weeks, they are then randomized to receive either diethylstilbesterol alone or in combination with chemotherapy. The patients relapsing on the hormone arm alone will subsequently receive the combination chemotherapy regimen but without the hormone. This study is expected to be activated in March 1973.

C. Second Recurrence Investigations. 6X1

This protocol involves patients with rapidly advancing disease and patients who have failed primary hormonal manipulation. They are randomized to receive either cyclophosphamide, 5-fluorouracil and adriamycin or cyclophosphamide, 5-fluorouracil and methotrexate. This study is expected to be activated in February or March 1973.

The Medical, Surgical, and Radiation Breast programs are discussing a variety of options relating to surgical vs. radiotherapeutic castration, adrenalectomy vs. hypophysectomy (medical and surgical for both), Phase I drug studies, and drug combinations which would be non-cross-resistant with the regimen in our other study areas.

II. Ancillary Studies

Each of these programs is designed to provide either a better understanding of the disease process or to evaluate procedures which can be utilized early in the course of the disease. Since the clinical program was formally activated in January 1973 there is as yet little useful information in any of these areas.

A. Randomization Techniques. Breast carcinoma is a disease which is associated with a large number of prognostic factors. These necessitate a large stratification matrix in order to provide balance in each cell when randomization is performed. The usual techniques of randomization only assure balance within each stratification cell.

In a two arm study it becomes possible to have two or three times as many patients on one arm as on the other, thus unduly prolonging the study. In cooperation with Dr. Richard Simon we have designed a system which attempts to avoid this problem while still providing balance of the regimens within each cell. This system, which we have termed an "updated balanced-cell procedure," is currently undergoing evaluation and appears to be headed for a system wherein a computer will be programmed for a series of cascading probabilities for each cell. When a new patient enters the study a printout would be provided for the regimen which the next one to two patients should receive. The randomization sheet is thereby updated each time a new entry occurs. This is an ongoing program aimed at refining our techniques. 8XX

B. Data Collection, Storage, Retrieval and Analysis

At present the usual method for performing clinically related studies involves hand collection of data from a patient's flow sheets and medical record with reference to each individual study. It would be considerably simpler if both the basic patient data and any individual study data could be placed on a computer file. Appropriate cross-indexing of this data would then allow numerous investigators to correlate their individual data with the patient's basic medical record. This would save innumerable data with the patient's records by a number of investigators, all of whom are interested in selected correlations. In cooperation with Dr. Richard Simon's group we have programmed our basic medical records and now are beginning to receive printouts of that information for the medical records. We are in the process of programming the patient log books, interim analyses of protocols, individual patient courses, and automatic cross-indexing to all other studies, such as hormone and tumor marker investigations, and epidemiologic variables. These programs will be constantly updated and refined and this will therefore be a continuing project. 8XX

C. Early vs. Late Radiotherapy for Osseous Lesions

One of the major clinical supportive care problems in breast cancer involves fracture and/or compression of involved bones. Traditionally these lesions are radiated either after the clinical problem occurs or when radiologic evidence suggests they will occur. With the increased sensitivity and accuracy of radionuclide scanning it has become feasible to ascertain if radiotherapeutic treatment of roentgenogram negative-scan positive lesions will prevent later problems. In cooperation with Dr. Thomas Pomeroy of the Radiation Branch we are discussing how best to integrate such a study into our program. 6X1

D. Chromosome Analysis

In cooperation with Dr. Jacqueline Whang-Peng and the Surgery Branch, we are attempting to provide material serially on our patients for chromosomal analyses. This project is designed to further our understanding of the disease process. To date no material has become available for this study. 6X1

E. Tumor Marker Analyses

We are making arrangements with Dr. T. Philip Waalkes' laboratory to supply sequential blood and urine samples from patients for analysis of tumor markers. These results will be correlated with the clinical cause of the disease. Since breast carcinoma patients commonly experience a disease-free interval they afford a unique opportunity to test both qualitative and quantitative sensitivities of such markers. 6X1

F. Pathologic Evaluation

We have arrived at a common breast tumor pathology analysis with the Surgery Branch and Dr. Gregory O'Conor of the Pathology Branch. This analysis supplies the usual diagnostic histologic information as well as evaluating the aggressiveness of the lesion and the host reaction to the lesion. 6X1

G. Radionuclide Scanning Procedures

The diagnostic importance of brain, liver-spleen, lung, marrow, bone and total body scans in breast carcinoma has not been evaluated systematically. We have established with the Radionuclide Branch a standard series of scans to be performed on a routine sequential basis in our patients. The results of these procedures will be correlated with the clinical state to ascertain their capabilities relative to other clinical modalities. It is hoped that these procedures will prove to be a more sensitive indicator of response and failure to therapy than roentgenograms, chemical, or physical examination procedures. 6X1

H. Hormone Analyses

We are attempting to establish contracts to assay blood and urine levels of pituitary hormones, androgens, estrogens, and corticoids. These would be performed at diagnosis and before and after therapeutic hormonal and chemotherapeutic manipulations. The results will be analyzed to determine if any of these hormones can be predictive for each therapeutic maneuver. 6X1

III. Extramural Activities

The activities listed below all have a direct bearing on the program of the Medical Breast Unit either in terms of providing patient material or in integrating the NCI programs across varying branches.

A. Contract Programs - 6X1, 7XX, 5XX

The National Naval Medical Center, Bethesda, and Walter Reed Army Hospital, Washington, are under contract to NCI. Douglass C. Tormey is Project Officer for these contracts. Both institutions take part in our protocol designs and utilization, thus shortening the duration of time required to complete our studies. To a lesser extent, they participate in some of our Ancillary Studies Programs. In addition, Walter Reed ArmyHospital runs Phase II and III protocols in other diseases, They are presently active in Hodgkin's disease and other lymphomas, acute leukemia, melanoma, lung, sarcoma, head/neck/cervix, squamous cell tumors, and cell cycling concepts.

Dr. Tormey has been working with Dr. Simon to increase the efficiency of data collection for improved analysis in the Phase II contracts of Dr. Stephen Carter. Newer computerized methods will be presented to the three contractors and discussed this spring.

B. Breast Cancer Task Force - 1XX, 7XX

The Medical Breast Unit participates on the Treatment Subcommittee of the Breast Cancer Task Force. Douglass C. Tormey is leading site visits to several centers to evaluate cell kinetic proposals. Our Unit has also been involved in writing announcements for proposals to treat Stage III disease, and first recurrences in pre- and postmenopausal patients. Our Unit is continually collecting data on randomized trials at all stages of breast carcinoma. These are being filed on a varying computer program and will be ready for continual updating and distribution to Task Force and NCI personnel by April 1973.

C. Cooperative Group Affiliations - 5XX, 5X1, 7XX

The Medical Breast Unit is affiliated with the National Surgical Adjuvant Breast Project (NSABP), the Eastern Cooperative Oncology Group (ECOG), and Acute Leukemia Group B (ALGB). We are presently participating in the NSABP-ECOG adjuvant chemotherapy protocol. Douglass C. Tormey has been appointed co-chairman of the Breast Tumor Subcommittee of ECOG, and has authored an adjuvant chemotherapy protocol for ALGB which is due to be activated. He is also proposing protocols for disseminated disease to both ECOG and ALGB.

This Unit's affiliation with these groups affords a unique opportunity to coordinate studies between these investigators.

Serial No. NCI-6701
1. Medical Breast Unit
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Factors Controlling Lymphocyte Reactivity in Vitro

Previous Serial Number: None

Principal Investigator: Douglass C. Tormey, M.D., Ph.D.

Other Investigators: Lynda Chiodetti

Cooperating Units: None

Man Years:

Total: 1.3
Professional: 0.2
Other: 1.1

Project Description: 1XX

Objectives:

The normal physiologic controls of organized cell replication appears to be deranged in tumor growth. In tumor tissue there appears to be a greater probability of cellular replication as expressed in an abnormally high growth fraction compared to the normal tissue. This increased replication probability can also be expressed as a tendency for cells to leave the nonreplicating G1 and G2 compartments to take part in one or more replication sequences. Physiologic "hormones" have been shown to inhibit (e.g., chalones) or stimulate (e.g., bone marrow colony stimulating factor) this process. The goal of this project is to attempt to isolate and identify similar entities for lymphocyte replication. The lymphocyte was chosen as a model for these interactions because human cells can be used, they can be purified, subpopulations can be identified, they exist in a prolonged G1 (?GO) state, can be selectively stimulated into parasynchronous growth moving sequentially through the cell cycle, the cell cycle transit times are known, and there is considerable background on the biochemistry of these cells following stimulation. The roles of lymphocytes in the immune response was an added attraction. In previous investigations my associates and I have identified a specific G1 requirement for transferrin by at least a subpopulation of these cells, confirmed the existence of an $alpha_2$-globulin containing fraction of human serum for these cells, shown that crude pig spleen extracts are also inhibitory, and demonstrated the existence of a growth factor which is released by lymphocytes within 3 hours of culture.

Methods Employed:

Since October our laboratory has been attempting to isolate and define the cycle specificity of these last three factors by various protein fractionation techniques and tissue culture assays.

Major Findings:

Work has been hampered by the necessity to wait for specific tissue culture equipment but from January to March 1973, the culture assays have been standardized; accordingly, we can now begin to test our fractions in vitro.

Significance to Biomedical Research and the Program of the Institute:

This project has broad applicability to problems of altered cell growth, especially cancer. The elucidation of molecular factors which physiologically control the differentiation of cells has direct potential application to cancer therapy.

Proposed Course:

This work is very preliminary and is continuing. A technician was brought into this program in October 1972.

Honors and Awards:

None.

Publications:

1. Tormey, D. C. and Mueller, G. C.: Biological effects of transferrin on human lymphocytes in vitro. Exper. Cell Res. 74: 220-226, 1972.

Serial No. NCI-3403
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical Trials and Miscellaneous Clinical Investigations

Previous Serial Number: Same

Principle Investigators: Vincent T. DeVita, George P. Canellos, Robert C. Young, Philip S. Schein, Bruce Chabner, Stephen H. Rosenoff, James C. Arseneau, Susan Hubbard

Other Investigators: Ralph Johnson, Joan Bull, Harvey Gralnick, Richard Sherins, Robert Sponzo, Oleg Selawry, Michael Milder, First Year Clinical Associates

Man Years:

Total:	11.25
Professional:	9.25
Other:	2.00

Project Description:

The Medicine Branch is a major clinical facility of the Cancer Institute. Its activities are divided between clinical therapeutic trials in patients with cancer, most of which are reported here under project No. 3403, and clinical research efforts aimed at understanding the natural history of the disease in question, the interaction of the drugs used to treat them with the host, and parallel phenomena associated with cancer, chemicals and the host. These latter studies are reported in companion projects No. 6500, Clinical Pharmacology; No. 3420, Kinetics of Cellular Proliferation; No. 3405, Hematology Studies, and No. 3404, Immunologic Aspects of Cancer.

The clinical studies are conducted by the 4.5 senior staff (includes the branch chief and one investigator, Dr. Chabner from the Laboratory of Chemical Pharmacology, spending 50 percent of his time with the Medicine Branch),one research nurse, and six first year Clinical Associates.

The clinical resources of the Branch are 19 beds and three outpatient afternoon clinics a week.

In the preceding year 34 percent of all NCI admissions were admitted to the Branch's 19 beds on Ward 12-West; these admissions constituted 25 percent of all new NCI admissions and 37 percent of all readmissions. A total of 4,388 outpatient visits occurred last year or 25 percent of the NCI outpatient visits.

The total census in the preceding year was 494 patients. Thirty percent had solid tumors primarily ovarian carcinoma, melanoma and breast carcinoma, 32 percent Hodgkin's disease, 28 percent histiocytic, lymphocytic or mixed lymphomas and 10 percent chronic leukemias.

In recent years the Branch has been attempting to increase the referral pattern toward patients with the solid tumors under study.

Clinical trials of the Medicine Branch involves two major areas:

1. New antitumor agents developed through the NCI program are evaluated for toxicity and therapeutic benefit in patients with otherwise untreatable metastatic cancer. (Phase I studies)

2. Disease Oriented Studies

   The Medicine Branch has a disease oriented therapeutic program. The following tumors are being intensively studied:

   1. Hodgkin's disease
   2. Histiocytic lymphomas (reticulum cell sarcoma)
   3. Lymphocytic lymphomas (lymphosarcoma)
   4. Burkitt's lymphoma
   5. Ovarian carcinoma
   6. Carcinoma of the breast
   7. Malignant melanoma
   8. Chronic myelocytic leukemia
   9. Osteogenic sarcoma

A. Phase I and II Studies

1. Adriamycin (V, 5XX, LA 100)

Adriamycin is an anthracycline antibiotic with clinical antitumor activity against a wide range of human solid tumors. The compound has been used in cancer patients as a secondary therapy after failure on conventional treatment programs. Clinical activity has been demonstrated in patients with breast cancer, lymphoma and sarcomas using a single intravenous dose of 60 mg/m^2 intravenously. The principal toxicity has been depression of white blood cell count with a relative span of platelets. The compound has now been incorporated into a new drug combination (BACOP) see below)

2. MeCCNU (V, 5XX, LA 100)

A phase I clinical trial of MeCCNU has been completed and the manuscript is in press for publication in Cancer. Preliminary clinical trials indicated that MeCCNU was well-tolerated and biologically active when given orally and could be given to previously treated patients at 200 mg/M^2 or less and to previously untreated patients at > 220 mg/M^2 but < 290 mg/M^2. Initial responses were seen in melanoma, primary and secondary brain tumors, Hodgkin's disease, lymphosarcoma and histiocytic lymphoma. Because of the initial good responses in several patients with widespread malignant melanoma, we have initiated a phase II trial of this agent in malignant melanoma.

Twenty-five patients with widespread melanoma have now entered the trial and 2/3 have gotten an initial dose of 250 mg/M^2. Some modification of dose is usually necessary with subsequent treatment, because of the cumulative toxicity of this agent first demonstrated in our phase I trial. Delayed bone marrow toxicity especially effecting platelets is the major dose limiting toxicity. To date, approximately 25% of patients have had a response to MeCCNU and there have been 4 patients with good partial remissions of > 50% of their followable disease. Skin, nodal, lung, and even bone lesions have been shown to regress. The phase II trial of MeCCNU in metastatic melanoma is continuing.

3. Procarbazine (V, 5XX, LA 100)

Twenty patients have received intravenous pulse doses of procarbazine in a phase I trial seeking information as to maximum tolerated dose of this agent given intermittently by the IV route. Significant toxicity in the form of prolonged lethargy and somnolence has been observed with doses of 2000 mg/m^2 or greater. It is anticipated that a safe IV dose for this agent will be approximately 1500 mg/m^2 IV on a weekly basis. This study is now completed.

4. Streptozotocin (V, 5XX, LA 100)

Streptozotocin is a nitrosourea antitumor agent with the unique property of having activity against the L1210 system without myelosuppression. A phase II study with streptozotocin has been conducted to determine whether this property can be exploited clinically.

Clinical antitumor activity has been demonstrated in patients with advanced refractory Hodgkin's disease and melanoma. No objective responses have been observed in 6 patients with malignant carcinoid.

The toxicologic properties of streptozotocin have been defined. Nausea and vomiting has been observed in all patients. Renal tubular toxicity is the major therapy limiting effect and can be anticipated by pretreatment measurements of urinary protein excretion. No myelosuppression has been observed. A return of bone marrow cellularity has been observed in patients on treatment who have severe myelosuppression prior to the initiation of treatment.

5. Hexamethylmelamine(HXM) (V, 5XX, LA 100)

Previous phase I studies have demonstrated HXM as a primary therapy has activity against ovarian and lung cancer without major bone marrow toxicity. Studies have been instituted to examine the antitumor activity of HXM in advanced alkylating agent resistant ovarian cancer to assess (1) the possible role of this agent in future combination treatment studies; (2) to determine whether cross-resistance exists between HXM and classical alkylating agents. Patients with ovarian cancer have been treated with initial doses of 8 mg/kg/day. One objective response has been observed in a patient whose tumor was refractory to phenylalanine mustard. This remission lasted approximately 3 months.

The principal clinical toxicities observed have been nausea, mild myelosuppression, and in one patient, neurologic toxicity consisting of agitation and peripheral neuropathy which was reversible with dosage reduction. This study continues.

B. Disease Oriented Programs

1. Ovarian carcinoma (IV, 6X1, LA100)

Ovarian carcinoma is the fourth most common killer of women among cancers and the incidence is increasing at the rate of 1% per year. The Medicine Branch became interested in the problem of ovarian cancer two years ago and extensively reviewed the subject in the New England Journal of Medicine in an article entitled, "The Treatment of Ovarian Carcinoma: Possibilities for Progress." We have begun a large scale study of the problem combining in-house, contract and laboratory based investigations. In-house studies are in progress in two phases. In collaborations with the Radiation Branch patients with stage II ovarian carcinoma (VII, 7XX, LA 100) are being randomly allocated to radiotherapy to the pelvis or radiotherapy plus chemotherapy in an attempt to prevent recurrences in this stage of the disease. Our in-house efforts with stages III and IV disease were initially directed toward a randomized trial between cyclic oral melphalan at 0.2 mg/kg p.o. q.o. x 5 days every 4-6 weeks, and high dose cyclophosphamide at 40 mg/kg IV q 12 hours, x 2 repeated every 4 weeks. Twenty-three patients were randomized.

Results are as follows:

Conventional Therapy	Intensive Therapy
CR - 15%	CR - 10%
PR - 46%	PR - 50%
NR - 38%	NR - 40%

The responses in the two groups were comparable but the toxicity of the intensive therapy arm was much more severe. We have, therefore, concluded that the intensive use of high dose cyclophosphamide offers no significant advantage over conventional therapy, and we have terminated that study in favor of a randomized trial between conventional Melphalan therapy and a new combination known as Hexa-CAF.

The new Hexa-CAF regimen is as follows:

	Day 1	8	14	28
5-FU	600 mg/M^2	600 mg/M^2		
MTX	40 mg/M^2	40 mg/M^2		
CTX	150 mg/M^2	→	→	
Hexamethylmelamine	150 mg/M^2	→	→	

This regimen is repeated at monthly intervals for 6 cycles.

Other in-house studies have included (a) initiation of a phase II effort with hexamethylmelamine, high dose procarbazine, and adriamycin in patients with refractory ovarian carcinoma (b) kinetic studies on patients with ovarian ascites tumor (c) the use of peritoneoscopy in the staging of ovarian carcinoma (d) the use of lymphangiography in the staging and followup of patients with ovarian carcinoma. Our goals in ovarian carcinoma are to extend the small but definite (5-10%) long term survival induced by chemotherapy and to assist in this task the Medicine Branch and the National Cancer Institute have embarked on a contractual arrangement with M.D. Anderson Hospital to identify antitumor activity of new and older drugs which might be useful in the treatment of ovarian carcinoma. The initial trial randomly allocates patients with advanced disease to either Melphalan, Hexamethylmelamine, or 5-FU. A crossover study will be employed to test for cross-resistances. Patients who fail on such regimens will then be treated with Phase I agents, the first of which will be MeCCNU.

2. Hodgkin's Disease (VI, 6X1, LA 100)

The study employing nitrogen mustard, vincristine, and procarbazine and prednisone (MOPP) for Hodgkin's disease continues to accrue patients. The group treated between 1964 and 1967 continues to be followed to assess recurrence rate and survival after complete remission. No relapses have occurred after 42 months of remissions; some of these patients

are now nine years from the end of treatment; 43 percent of the original group of complete responders remain in their first remission.

In 1967 a trial was instituted randomizing patients achieving complete remissions to either no therapy (control), intermittent BCNU therapy or intermittent MOPP maintenance, the latter two therapies given at 3 monthly intervals for a total of 15 months.

This study has now been completed with a total of 90 patients entered with the following results and conclusions. The complete remission rate in this group (75%) is comparable to the original group (81%). Relapse rates in the three groups control (24%) MOPP (25%) and BCNU (16%) were not significantly different from one another, although those receiving no therapy relapsed sooner. Because these patients are easily retreated, there is no difference in survival at this point in time.

The five year survival of the complete responders in this study is 86% with a median duration of remission of 48 months. Using the worst slope of the survival curve a projected median survival for the entire group of complete responders is 19 years. It should be emphasized that although this is a projected figure, many of the patients in the original study are approaching 9 and 10 years from the end of treatment. Using hard data, the median survival in this group of complete responders will be well in excess of 7 years. In light of this observation, the above data seems like a reasonable estimate.

In 1969, patients with stage IIIB disease only were assigned to therapy with 6 cycles of MOPP followed by total nodal irradiation.

Thirty-three patients have been entered on study which is not yet completed. Preliminary conclusions are that (1) such an approach is complicated and difficult to complete successfully, (2) The permanent morbidity may be high including an increased risk of developing second malignancies. (See below) In spite of these observations, the recurrence rate of Hodgkin's disease may be far less than our previous experience at NCI with stage IIIB patients. Current plans are to combine MOPP plus x-ray to the dominant sites of relapse in patients with stage IIIB and IV disease to attempt to prevent recurrences in high risk patients.

3. Lymphocytic and Histiocytic Lymphoma

a. Chemotherapy (VI, 6X1, LA100)

The results of CVP and MOPP combination chemotherapy programs in the treatment of 80 patients with advanced non-Hodgkin's lymphoma have been correlated with the Rappaport classification system. Thirty-six patients (45%) achieved a complete remission. Twenty-eight percent of all patients and 57% of complete remitters remain free of disease 1 to 7 years with a projected median duration

of remission in this group of 3 and 1/2 years.

Significant differences in prognosis were found between histologic groups. Well-differentiated and nodular histology were positive determinants for response and survival. One-third of patients with advanced diffuse histiocytic lymphomas achieved complete responses and none have relapsed. Failure to achieve a complete response was associated with early demise.

b. Randomized trial between radiotherapy and chemotherapy in the Non-Hodgkin's lymphomas (VI, 6X1, LA100)

Favorable therapeutic results for patients with advanced non-Hodgkin's lymphomas have been reported using total nodal and total body X-irradiation from the NCI's Radiation Branch. Because patients with apparently equivalently advanced disease were also doing well with combined chemotherapy, a controlled trial was established between the Medicine and Radiation Branches to determine the relative efficiency of each therapy by stage and histologic subtypes.

After an extensive staging evaluation patients are randomized by stage, and histology to various treatment programs as follows:

1. Histiocytic and Mixed Lymphomas

   Stage I, II -- Local radiotherapy or local radiotherapy followed by C-MOPP
   Stage III -- Total nodal radiotherapy or C-MOPP

   Stage IV -- BACOP Therapy (See below)

2. Lymphocytic Lymphomas

   Stages I and II -- Local radiotherapy only

   Stage III -- Total nodal plus total body irradiation versus cytoxan, vincristine and prednisone (CVP therapy)

   Stage IV (also randomized by site of organ involvement) Total body irradiation plus irradiation to dominant disease versus CVP therapy

To date, 75 patients have been randomized. Although no apparent difference in survivals has appeared in any group as yet, the results are still too preliminary for analysis and the study is continuing to accrue patients.

c. Evaluation of new drug combination (BACOP) in advanced histiocytic lymphoma (VI, 6X1, LA 100)

Despite the ability of some patients with stage IV diffuse histiocytic lymphoma to achieve a complete remission and long survival, the overall median

survival is 6 months after initiation of chemotherapy since less than half of patients can be rendered free of disease. Patients have demonstrated a deceivingly favorable initial response to treatment which is followed by a pattern of rapid relapse during recovery phases of MOPP chemotherapy cycles.

The BACOP protocol takes into account these clinical observations and makes two major changes in the previously used MOPP protocol: adriamycin, a drug with a reported response rate of 50% as a single agent in this disease, is substituted for procarbazine; prednisone and bleomycin, two non-myelosuppressive agents, are employed during the second half of the 28 day cycle in an attempt to retard the regrowth of disease prior to the initiation of the next cytotoxic portion of the regimen.

Six patients, three previously treated with chemotherapy or radiotherapy, have been treated or are currently on treatment. Two patients have completed six cycles of BACOP chemotherapy and are in complete remission.

4. The American Burkitt Lymphoma Registry (VI, 6X1, LA 100)

Burkitt's lymphoma (BL), the most common childhood tumor of tropical Africa, is now being recognized with increasing frequency within the United States. Although a relatively rare tumor, in comparison to Hodgkin's disease, this tumor is of particular importance to oncologists, because of the epidemiologic data indicative of a viral etiology. The observation that American BL patients exhibit many of the clinical, epidemiological, and virological features of the African cases has led to an equally intensive attempt to investigate the disease in this country as well through the development of an American Burkitt Lymphoma Registry. The absence of malaria and the opportunities for careful patient followup in the United States have provided an opportunity for the study of an important comparative group which can be compared to African BL. As an extension of the original Medicine Branch study the clinical features of the 97 patients confirmed by the registry in its 24 months of operation and survival data up to February 28, 1973 were collated.

It has been possible to trace 11 patients who are still alive and off of therapy for more than 3 years, and four of these are free of disease between 5 and 10 years from the time of diagnosis. Of the 21 NIH cases diagnosed between 1965 and 1970, 8 [38%] are still alive as compared with 16 [33%] of the 48 non-NIH cases.

The wide variety of drugs used in these cases made analysis of survival on the basis of therapy impossible, but the treatment given to long-term survivors, noted in Table VI, does not reveal any specifically effective regimen. Although 62% of the patients failed to exceed a one year survival, the sensitivity of the tumor to therapy was indicated by the fact that 75% of the 36 patients on whom information on drug response was obtained were

reported to have a more than 50% reduction in tumor size with the first course of therapy.

5. Combination drug Therapy of Advanced Breast Carcinoma (VI, 6X1, LA 100)

Advanced cancer of the breast cyclic combination chemotherapy has been studied in metastatic breast cancer refractory to hormones. Twenty-four patients have been entered on a protocol consisting of 5-FU 700 mg/M^2 IV and Mtx 60 mg/m^2 IV are given on day 1, 8 with continuous daily oral cytoxan 100 mg/m^2 and prednisone 40 mg/m^2 for 14 days. Days 14-28 are left free of drug. Out of 23 evaluable patients, 16 (70% had a clinically useful response with 7 complete (30%) and 9 partial responses. Our of this group of patients stratification in response occurred.Of various sites involved by tumor 5/5 nodal areas, 5/11 lung lesions, 2/4 soft tissue had a complete regression. Responders had 10 cycles of chemotherapy with a median duration of response of 6+ months (2-18); 14/16 responders are living; all nonresponders are dead in a median of 6 months. The toxicity was tolerable with marrow suppression as the main side-effect.

6. Melanoma Adjuvant Study (VII, 7XX, LA 100)

A cooperative trial is now being initiated in conjunction with the Surgery Branch and the Immunology Branch, NCI in which the merits of adjuvant immunotherapy or chemotherapy are being tested after surgery in patients with stage II melanoma at high risk of relapse. Patients will be randomized after definative surgery to receive either chemotherapy (MeCCNU 200 mg/M^2 p.o. q 6 wks) or immunotherapy consisting of lethally irradiated neuronidase treated cells and BCG. Patients will be treated for 1 year and then followed for rate of relapse and survival.

Patients with more advanced stage III disease will also be randomized to receive either immunotherapy or chemotherapy with the same regimens and will be followed for remission rate and survival.

7. Osteogenic sarcoma adjuvant study (VII, 7XX, LA 100)

Only 15-20% of patients undergoing presumed curative surgery for osteogenic sarcoma are alive at 5 years. Recently the antibiotic adriamycin plus dimethyl triazeno imidazole carboxamide (DTIC) have been demonstrated to be clinically active in advanced stages of the disease. An adjuvant project has been designed to study the ability of adriamycin or the adriamycin DTIC combination in preventing relapses in patients who have had resection of all clinical evidence of disease. This project has just been initiated.

8. Streptozotocin in the treatment of malignant insulinoma (V, 5XX, LA 100)

Six patients with advanced malignant insulinoma have been treated with intravenous streptozotocin using a single weekly dose or daily treatment for five consecutive days. One patient has had an objective response which lasted for one year. There were no distinguishing features of this case that would predict for response other than the relatively small amount of hepatic metastases. The remaining patients have not had useful therapeutic responses.

C. Clinical Investigations

1. Staging in lymphomas (VI, 6X1, LA 50, 5A 50)

The natural history of the non-Hodgkin's lymphomas varies markedly from Hodgkin's disease, a not greatly appreciated fact. They are rarely localized even when they appear to be and they spread unpredictably. Much of the country is applying the same staging procedures used in Hodgkin's disease to the non-Hodgkin's lymphomas an approach we consider erroneous.

To evaluate the proper approach to staging, we have conducted an extensive study using serial biopsy procedures up to and including laporatomy and splenectomy, if necessary. The comprehensive analysis of findings in approximately 100 untreated patients with non-Hodgkin's lymphoma has been completed. This study indicates that 70% of patients had Stage IV disease after undergoing a sequence of staging procedures that included bone marrow biopsy, routine liver biopsy, and peritoneoscopy. Laparotomy was performed in the remaining 30% of patients to rule out intraabdominal disease, and disclosed liver involvement or positive mesenteric nodes in an additional 20% of patients. Thus 90% of patients were found to have disease inaccessible to high dose radiotherapy; these patients were, therefore, spared unnecessary intensive radiotherapy. We conclude that laparotomy can be avoided in most patients with non-Hodgkin's lymphomas, if routine biopsy procedures are performed.

2. Peritoneoscopy (VI, 6 X1, LA 50, SA50)

We have developed the use of the peritoneoscope as a medical oncology tool. This diagnostic procedure has been particularly useful as an alternative to staging laparotomy in Hodgkin's disease. Based on our old data suggesting peritoneoscopy is quite as effective as laparotomy and biopsy to identify tumor in the liver, we have initiated a sequential study in patients with clinical stage IIIB, Hodgkin's disease to test the sensitivity of a negative peritoneoscopic examination by subsequent laparotomy and biopsy.

Peritoneoscopy is also being employed in initial staging in patients with ovarian carcinoma and is used as a second look procedure in those patients who achieve remission after drug therapy.

3. Study of reproductive capacity on men after intensive chemotherapy (VI, 6X1, SA 100)

We have previously shown that intensive drug therapy results in male sterility in 75% of patients. Reproductive function was evaluated in 16 men who have been treated with MOPP, MOMP, CVP or cyclophosphamide for lymphoma, were in remission and had received no therapy for 6 months to 7 years. Although libido and potency were normal in all patients, ten men were azoospermic and testicular biopsy demonstrated complete absence of germ cells;two men showed minimal evidence of spermatogenesis; and four men in remission 2-7 years, demonstrated complete spermatogenesis at the time of their evaluation. Although there was considerable overlap in the duration of remission, seemingly required for return of spermatogenesis, within each treatment group the men with evidence of normal germinal tissue were those men who were in longest remission for that group. These data suggest that spermatogenesis may be reinitiated after extensive chemotherapy but that it is likely to occur more than two years after drug treatment. Similar studies are in progress in women who have been successfully treated.

4. Bone Marrow Involvement in Hodgkin's disease (VI, 6X1, LA 100)

A study of the bone marrow findings in untreated patients with Hodgkin's disease admitted to the NCI between 1965 and 1972 has been completed. The pathology of this lesion was clarified, including the constant relationship between tumor involvement and myelofibrosis, and the reversibility of reticulin fibrosis with therapy was documented in eight of 15 patients with this complication. A favorable prognosis was found for patients with marrow involvement treated with MOPP chemotherapy, as 10 to 15 patients have been in continuous remission for a median duration of 30+ months extending up to 8 years.

5. Second Malignancies in Hodgkin's disease (VII, 7XX, L A 100)

The study of second malignant tumor development in Hodgkin's disease and its relation to therapy has been completed and published. A further clinical study has been designed to intensively investigate those patients found to be at high risk for new neoplastic disease in an attempt to identify early or preneoplastic lesions and possibly, by these means, improve therapy.

6. Protocol for the study of cachexia in malignancy (VII, 7XX, EA 100)

The cachectic state in patients with malignancy is poorly understood and occasionally irreversible, even when an antitumor effect has been achieved. Because of recent interest in the use of hyperalimentation in patients unable to consume sufficient calaries to maintain body weight, a study has been initiated to examine metabolic derangements in cachectic patients.

7. Metabolic Complications of Chemotherapy in Burkitt's Lymphoma (VII, 7XX, SA 100)

Hyperkalemia developing within 48 hours of chemotherapy was found in retrospective analysis in 5 of 22 evaluable cases of Burkitt's lymphoma patients at the NIH. This syndrome was fatal in 2 of these 5 patients and was the suspected case of sudden post-chemotherapeutic death in 2 further cases in which post-chemotherapeutic chemistries were not obtained.

Clinical and animal studies suggest that hyperkalemia is related to sudden lysis of large volumes of tumor. Patients at series risk were found to be those with large tumor masses and/or impairment of renal function.

Close observation, parenteral hydration and diuretic therapy, control of hyperuricemia, reduction of the initial dose of chemotherapy, and if necessary, dialysis are suggested during the initial therapy of these high risk Burkitt lymphoma patients.

8. Regan Isoenzyme is a Marker in Patients with Ovarian Carcinoma (V, 5XX, LA 100)

An isoenzyme of alkaline phosphatase has been shown to be produced by a number of tumors particularly those originating from the ovary. A spectrophotometric measurement of the Regan isoenzyme of alkaline phosphatase is being evaluated as are indicators of increased levels of this enzyme related to tumor volume in ovarian carcinoma. In conjunction with Dr. Phillip Waalkes, a radioimmunoassay is being developed to provide greater sensitivity for smaller volumes of tumors.

Honors and Awards:

Dr. DeVita

1. Albert and Mary Lasker Foundation Medical Research Award, November 1972.

2. Visiting Professor of Medicine, Stanford University School of Medicine, July 1972.

3. "The Chemical Control of Cancer," presented to the American Chemical Society of Washington, D. C., December 1972.

4. "Chemotherapy of the Patient with Hodgkin's disease," presented at Conference on Coordinated Cancer Therapy, St. Mary's Long Beach Hospital, Long Beach, California, 1972.

5. Participant on Panel, "The Modern Management of Hodgkin's disease," at New York Regional Meeting of American College of Physicians, Rockefeller University, New York, March 1973.

6. "The Management of Lymphomas," presented at the Meeting of the Tumor Registrars Society of Metropolitan Washington, D. C., March 1973.

7. "Lymphoma," presented at Symposium on The Modern Treatment of Cancer, sponsored by Academy of Medicine of New Jersey College of Medicine of the New Jersey College of Medicine, and the Newark Beth Israel Medical Center, April 1973.

8. Participant on Panel on "Lymphoma" at 54th Annual Session of American College of Physicians, Chicago, April 1973.

9. Medicine 101 Lecture Series. Department of Medicine, George Washington University School of Medicine, Lecture on Hodgkin's disease, March 1973.

10. "Ovarian Carcinoma," presented at Medical Grand Rounds, Department of Medicine, Stanford University School of Medicine, Palo Alto, California, July 1972.

Dr. Canellos

1. "Treatment of Lymphoma," presented at Educational Panel, American Society of Hematology, Hollywood, Florida, December 1972.

2. "Malignant Lymphomas," presented at Puerto Rican Society of Hematology, November, 1972.

3. "Malignant Lymphomas," presented at University of California Graduate Symposium, March 1973.

4. "Malignant Lymphoma," and "Chemotherapy of Sarcoma; metastatic tumor to the brain," presented at U.C.L.A. Postgraduate Course in Modern Concepts in Oncology, January 1973.

5. "Malignant Lymphoma: Natural History and Treatment," presented at Radiotherapy Division Lecture, University of Maryland, March 1973.

6. "Breast Cancer," Lecture at George Washington University School of Medicine, March 1973.

7. Moderator and Discussant on Workshop "Management of Advanced Breast Cancer," presented at American Society of Clinical Oncology Annual Meeting, Atlantic City, N.J., April 1973.

8. "Breast Cancer," Lecture at Walter Reed Army Hospital, Washington D.C., March 1973.

Dr. Young

1. "The Treatment of Ovarian Carcinoma," presented at Chemotherapy Conjoint Meeting, NCI, Annapolis, Md., Jan. 1973.

2. "Ovarian Carcinoma: Possibilities for Progress," presented at Cornell University Medical College, Jan., 1973.

3. "Ovarian Carcinoma: Current Status," presented at National Cancer Institute of Canada Meeting, Toronto, Ontario, Feb., 1973.

4. "Delayed Hypersensitivity in Hodgkin's disease," presented at International Society of Hematology, Sao Paulo, Brazil, July 1973.

5. "Chemotherapy of the Lymphomas," presented at 7th National Cancer Congress, Los Angeles, California, September, 1972.

6. "Lymphoma and Reticulum Cell Sarcoma," presented at Hematology Review Course, Walter Reed Army Medical Center, October 1972.

7. "The Management of Hodgkin's disease," presented at Harrisburg Hospital, Harrisburg, Penn., November, 1972.

8. "Hodgkin's Disease in Childhood," presented at Washington, D. C. Children's Hospital, November, 1972.

9. "Hodgkin's Disease and the Lymphomas, presented at New York Cornell Medical Center, January, 1973.

10. "The Treatment of Advanced Hodgkin's disease," presented at National Cancer Institute of Canada, Toronto, Canada, February, 1973

11. "Chemotherapy and Combination Therapy of Hodgkin's disease," presented at Cleveland Clinic Education Foundation, March, 1973.

12. "Chemotherapy in Non-Hodgkin's Lymphoma, presented at Cleveland Clinic Education Foundation, March 1973.

13. "Hodgkin's Disease in Childhood," presented at American Oncologic Hospital, Fox Chase, Penn. March, 1973.

14. "Maintenance Therapy in Advanced Hodgkin's disease," presented at Washington University Barnes Hospital, March 1973.

15. "Chemotherapy in Advanced Hodgkin's disease, " presented at Medical Group Teaching Day, May 1973.

16. "Maintenance Therapy in Advanced Hodgkin's disease in Remission: A Cautionary Note," presented at American Society of Clinical Oncology

Atlantic City, New Jersey, April 1973.

17. "Hodgkin's Disease- Current Therapeutic Management," presented at Seventeenth Annual Cancer Symposium, Saskatchewan, Canada, May, 1973.

18. "Treatment of Lymphoma," presented at Educational Panel, American Society of Hematology, Hollywood, Fla., December, 1972.

Dr. Schein

1. "Use of Chemotherapy in Carcinoma of Breast," and Chemotherapy of Ovarian Cancer," presented at Graduate Medical Symposium on New Drugs in Chemotherapy, Elizabeth City, N.C., January, 1973.

2. "Chemotherapeutic Management of the Hormone-Secreting Endocrine Tumors," presented at National Cancer Chemotherapy Conference, June 1972.

3. "Relationship of Chemotherapy to Second Tumors," presented at Late Effects Workshop Meeting, Childrens Cancer Research Foundation, Boston, Mass., October 1972.

4. "The Chemotherapeutic Management of Ovarian Cancer," presented at Medical College of Georgia, March 1972.

5. "The Potential for Long Survival with Chemotherapy in Non-Hodgkin's Lymphoma," presented at American Society of Clinical Oncology Annual Meeting, Atlantic City, N.J. April 1973.

6. "Chemotherapy of the Non-Hodgkin's Lymphomas," presented at The Johns Hopkins Hematology Division, March 1973.

7. "Chemotherapeutic Management of the Non-Hodgkin's Lymphomas," presented at Georgetown University Hematology Division, November 1972.

8. "Current Concepts in Chemotherapy," presented at Walter Reed Medical Oncology Seminar, October 1971.

9. "Current Concepts in Chemotherapy," and "Chemotherapy of Lymphomas," presented at XIV Reuniao Anual De Cancerologia, Sao Paulo, Brazil, Dec., 1972.

10. "The Non-Hodgkin's Lymphomas," presented at National Cancer Institute of Brazil Oncology Conference, Dec., 1972.

11. "Concepts in the Chemotherapy of Lymphomas," and "New Antitumor agents and New Concepts in Clinical Toxicity," presented at Professor,

Curso International de Cinetica Celular y Quimioterapia antineoplasica, Buenos Aires, Argentina, March 1973.

12. Lymphoma Panel, American Society of Hematology, Education Program, December 1972.

13. "Clinical Toxicity of Antitumor and Immunosuppressive Agents," presented at National Meeting of the American Medical Association, 1973.

14. "Current Status of Cancer Chemotherapy," presented at American Cancer Society, Nurses Seminar on Cancer, March 1973.

15. "Medical and Nutritional Factors Affecting the Cancer Patient," presented at District of Columbia Dietetic Association, March 1973.

Dr. Arseneau

1. "Recently Recognized Complications of Cancer Chemotherapy," presented at Conference on the Paraneoplastic Syndromes, sponsored by the N.Y. Academy of Science, New York, N.Y.

Publications:

1. Arseneau, J.C., Bagley, C., Anderson, T., and Canellos, G.P.: Hyperkalemia, a sequal to the chemotherapy of Burkitt's lymphoma. Lancet 1: 10-14, 1973.

2. Arseneau, J.C., Canellos, G.P., Sherins, R.J., and DeVita, V.T.: Recently recognized complications of cancer chemotherapy. Ann. N.Y. Acada. Sci. (In press)

3. Arseneau, J.C., Sponzo, R.W., Levin, D.L., Schnipper, L.E., Binner, H., Young, R.C., Canellos, G.P., Johnson, R.E., and DeVita, V.T.: Nonlymphomatous malignant tumors complicating Hodgkin's disease: Possible association with intensive therapy. NEJM 287: 1119-1122, 1972.

4. Bagley, C.M., Young, R.C., Canellos, G.P., and DeVita, V.T.: The treatment of ovarian carcinoma: Possibilities for Progress. New Eng. J. Med.287: 856-862, 1972.

5. Bagley, C.M., Young, R.C., Schein, P.S., Chabner, B.A., and DeVita V.T.: Ovarian carcinoma metastatic to the diaphragm: Frequently undiagnosed at laparotomy. Amer. J. Ob. and Gyn.(In press)

6. Schein, P.S., Vickers, H.R.: Lupus Vulgaris and Hodgkin's disease. Arch Derm. 105: 244-246, 1972.

7. Morganroth, J., Deisseroth, A., Winokur, S., and Schein, P.: Differentiation of carcinomatous and bacterial meningitis. Neurology (In press)

8. Schein, P.S.: Cancer Chemotherapy: Current concepts and results in Current Concepts in Oncology, Academic Press (In press)

9. Schein, P.S.: Chemotherapeutic management of the hormone-secreting endocrine malignancies. Cancer 30: 1616-1626, 1972.

10. Schein, P. S., Kahn, R., Gorden, P., Wells, S., DeVita, V.T.: Streptozotocin treatment of malignant insulinomas and carcinoids: report of eight cases and review of the literature. Arch. Int. Med. (In press).

11. Schein, P.S., DeLelis, R.A., Kahn, R., Kraft, A., Gordon, P.: Current Concepts and Management of islet cell tumors. Ann. Int. Med. (In press).

12. Sherins, R.J., and DeVita, V.T.: Drug therapy of lymphomas: The effect of treatment on the reproductive capacity of men in remission after therapy. Ann. Int. Med. (In press)

13. Young, R.C., DeVita, V.T., and Johnson, R.C.: Hodgkin's disease in childhood. Blood (In press)

14. Young, R.C., and DeVita, V.T.: Treatment of Hodgkin's disease. Current Therapy (In press)

15. Bagley, C.M., Thomas, L.B., Johnson, R.F., Chretien, P.R., and DeVita, V.T.: Diagnosis of liver involvement by lymphoma: Results in 96 consecutive peritoneoscopies. Cancer (In press)

16. Young, R.C., Walker, M.D., Canellos, G.P., Schein, P.S., Chabner, B.A., and DeVita, V.T.: Initial clinical trials with methyl CCNU 1-(2-Chloroethyl)-3-(4-methyl cyclohexyl-1-nitrosourea. Cancer (In press)

17. Chabner, B.A., Canellos, G.P., Olweny, C.M., and DeVita, V.T.: Late recurrence in testicular tumor. New Eng. J. Med. 287: 413, 1972.

18. DeVita, V.T., and Schein, P.S.: The use of drugs in combination for the treatment of cancer. New Eng. J. Med. 287: 413, 1972.

19. DeVita, V.T., Canellos, G.P., and Moxley, J.H., III: A decade of combination chemotherapy for advanced Hodgkin's disease. Cancer 30: 1495-1504, 1972.

20. Milder, M.S., Larson, S.M., Bagley, C.M., DeVita, V.T., and Johnston, G.S.: The liver spleen scan in Hodgkin's disease. Cancer (In press)

21. Bobrow, S.N., Jaffe, E., and Young, R.C.: Anuria and acute tubular necrosis associated with Gentanycin and cephatothin. JAMA 222: 1546-1547, 1972.

22. Mulvihill, J.J., Eckman, W.W., Fraumeni, J.F., Dryden, R.M., and Young, R.C.: Hodgkin's disease and Corneal keratopathy in a patient with the Melkerson Rosenthal Syndrome. Arch Int. Med. (In press)

23. DeVita, V.T., Canellos, G.P., Schein, P.S., Chabner, B.A., Bagley, C.M., and Young, R.C.: Malignant Lymphoma treatment with combination chemotherapy. Proc. 7th Nat. Cancer Congress. (In press).

Serial No. NCI-3404
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunologic Aspects of Cancer

Previous Serial Number: Same

Principle Investigators: Robert C. Young, Vincent T. DeVita, Philip S. Schein, Bruce Chabner, Stephen H. Rosenoff

Other Investigators: Paul H. Levine, John E. Bennett

Cooperating Units: Clinical Investigations Branch, NIAID, Viral Leukemia and Lymphoma Branch

Man Years:

Total	2.25
Professional:	1.00
Other:	1.25

Project Description:

A. To study the immunologic status of patients with malignancies and relate these findings to progression or arrest of the disease and survival.

B. To exploit the host's immunologic system as an adjuvant to chemotherapy of tumors.

C. To exploit antigenic markers in the therapy of patients with cancer.

D. To identify, study and develop therapy for infectious complications of patients with cancer.

A. Immunology and Cancer

1. Immunology of Hodgkin's disease (VI, 6x2, EA100)

a. A comprehensive study on immune alterations in Hodgkin's disease has been published this year, and the details have been reviewed in previous Annual Reports. Because the nonspecific tests of general immunologic status have not proven helpful in prognosis, current studies are now underway investigating EBV titers as well as tumor cell membrance extracts containing EBV associated and EBV negative antigens.

b. Interest has also centered around the role of Transfer Factor (VII, 7 XX, EA100) in patients with Hodgkin's disease. Patients with Hodgkin's disease seem to have a unique defect in their inability to respond to transfer factor. Since the defect in delayed hypersensitivity seems to be relative rather than absolute, we have begun testing patients with massive doses of Transfer Factor to see if it is possible to confer delayed hypersensitivity in Hodgkin's disease patients prior to therapy.

c. A comprehensive reevaluation of the immune status of patients who enter remission is now underway and includes retesting patients for delayed hypersensitivity after they achieve complete remission. Thus far, approximately 60% of those patients anergic before therapy have recovered the ability to respond to a skin test antigen after therapy. Studies to see whether they can now respond to Transfer Factor are now underway. Evidence thus far accumulated would suggest that the immunologic defect present with Hodgkin's disease has been eliminated by successful chemotherapy.

2. Immunologic Studies in Non-Hodgkin's Lymphomas (VII, 7XX, SA25, EA75)

Use of serum and skin testing to determine the relationship of prior exposure to E-B virus and subsequent development of non-Hodgkin's lymphomas. Studies of EB virus titers in serum in Hodgkin's disease have demonstrated a correlation with lymphocyte depleted histology. A prospective study investigating EBV titers as well as skin reactivity to tumor cell membrane extracts containing EBV associated and EBV negative antigens is being conducted in all histologic forms of non-Hodgkin's lymphoma to determine whether an association exists.

3. Tumor Specific Antigen in Ovarian Carcinoma (V, 5XX, LA100)

Associated with the recent clinical interest in ovarian cancer, samples of human ascites tumor have been grown in cell culture, and a program of antigenic extraction and animal immunization is underway to produce high titer specific antibody.

4. Pyridoxine Metabolism and the Anergic State (VII, 7XX, EA100)

A protocol was approved by the Clinical Research Committee of the NCI and the Medical Board of the NIH for studies of the role of pyridoxine deficiency in producing anergy in man. The proposed studies which are designed to elucidate the effects of pyridoxine deficiency on cellular and humoral immunity and various biochemical parameters in human normal volunteers, will begin in June of 1973. These studies are an extension of previous branch research in B_6 metabolism which identified a high frequency of B_6 deficiency in cancer patients in an anergic state. The

goal is to determine if progressive immunologic paralysis in patients with advancing cancer is a metabotic abnormality which is potentially reversible.

5. HLA Antigens in Hodgkin's Disease (VII, 7XX, EA100)

The HLA antigen system is a major histo-compatibility locus in man. Over 25 different HLA antigen s have been identified. Susceptibility to some leukemogenic viruses are clearly related to the alleles of the major histo-compatibility locus in several animal species. An examination of HLA antigens in a series of patients with Hodgkin's disease has been completed.

The results suggest that disease susceptibility and subsequent histologic pattern may be influenced by factors related to the HLA pheno-type of the host. A more recent observation of the waxing and waning of the expressed HLA antigens in patients at varying time intervals is being explored.

B. Infections and Cancer

1. Pneumocystis Carinii (VI, 6X1, SA50, EA50)

Investigations into the identification and treatment of this illness and our published results have been summarized in the Annual Reports, 1969-1972. The current study program involves a randomized trial of known or presumed Pneumocystis infections to either Pentamidine or Pyrimethamine and Sulfadiazine. Results thus far indicate that both regimens are effective in the disease but both have toxicities and problems associated with their administration. Data on the activity, toxicity and the utility of these two regimens will be of considerable aid in managing this previously uniformly fatal infectious complication of malignancy.

2. Aspergillus fumigatus (VI, 6X1, SA25, EA75)

Investigations into the patterns of infection, the immunology and the types of Aspergillus causing human infections have been reviewed in the Annual Reports 1970-1972. Plans are now underway in cooperation with the Laboratory of Clinical Investigation, National Institutes of Allergy and Infectious Diseases to sponsor a cooperative study of serological responses to Aspergillosis. Approximately 5 major institutions around the country will contribute sera from patients and each sera will be studied at each institution. At the completion, it will be possible to summarize the utility of serological testing in this frequent fungal complication of patients with malignancy.

Honors and Awards:

Dr. Young

1. "Aspergillosis," presented at University of Virginia, Department of Infectious Disease Seminar, November, 1972.

2. "Pneumocystis Carinii," presented at Grand Rounds, Washington Veterans Administration Hospital, February, 1973.

3. "Opportunistic Infections in Patients with Malignancy," presented at Grand Rounds, George Washington University, School of Medicine, April, 1973.

4. "Immune Alterations in Hodgkin's Disease," presented at Johns Hopkin's Medical Center, March, 1973.

5. "Delayed Hypersensitivity in Hodgkin's Disease," presented at International Society of Hematology, Sao Paulo, Brazil, July, 1972.

6. "Transfer Factor and Hodgkin's Disease," presented at meeting on Transfer Factor, Tuscon, Arizona, February, 1973.

Publications:

1. Young, R.C., Corder, M.P., Berard, C.W., and DeVita, V.T.: Delayed hypersensitivity and lymphocyte transformation in Hodgkin's disease. The effect on Course and survival. Arch. Int. Med. 131:446-454, 1973.

2. Graff, K.F., Simons, R.M., Yankee, R.A., DeVita, V.T., and Rogentine, G.N.: HLA antigens in Hodgkin's disease. Tissue Antigens (In press)

Serial No. NCI-3405
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Hematology Studies

Previous Serial Number: Same

Principle Investigators: George P. Canellos, Vincent T. DeVita, Jacqueline Whang, Philip Schein, Bruce Chabner, Robert C. Young

Other Investigators: Tom Anderson, James Arseneau, Gerald Johnston, Louis Gelrud

Cooperating Units: Surgery Branch, NCI; Nuclear Medicine Branch, Clinical Center, NIH

Man Years:

Total:	1.75
Professional:	1.00
Other:	0.75

Project Description:

The bone marrow is the major normal target tissue for most currently effective antitumor agents. Most drug scheduling and drug combination designs have been based on anticipated effects of drugs on the bone marrow and the time interval necessary to allow bone marrow recovery between treatment.

As such, the study of bone marrow function either by direct study of the marrow or assessed by alterations in number and function of peripheral blood elements is an integral part of medical oncology. In this project, studies in progress related to bone marrow are detailed along with the studies on one disease, chronic myelogenous leukemia, unique in its transition through a premalignant phase to a highly malignant disease in the presence of a specific identifiable chromosome marker.

A. Cytogenetic studies (V 5XX, LA25, SA50, EA25)

Collaboration with Dr. J. Whang-Peng has continued in an attempt to define a correlation between cytogenetic findings during blastic transformation and response to specific therapy. This represents the most completely studied groups of patients from diagnosis through to blastic crisis. Some of the more significant correlations are included under the discussion of blastic crisis therapy.

B. Therapeutic trials - CML

1. Chronic Phase Chemotherapy (VI, 6X1, SA50, EA50)

Forty patients have been randomized between dibromomannitol (DBM) and busulfan in order to investigate the comparative effectiveness of the former drug, a relatively new agent, in a group of previously untreated, Philadelphia chromosome positive recently diagnosed patients. The study has been completed and has required 5 years to properly evaluate this new agent in CGL. There were 16/20 complete remissions with DBM and 14/20 with busulfan. Failures were due to early blastic crisis and severe hypoplasia in 2 cases treated with busulfan. Mean time to remission was shorter with DBM (72 days) vs. 100 days for Busulfan. Busulfan resulted in a more prolonged myelosuppressive effect such that 9/14 remitters required a median time of 9 months before drug could be readministered for maintenance compared with only 3/16 DBM patients who required a delay. Extensive histologic evaluation revealed no differences in the extent of cellular atypia produced in lung or bladder tissue. The survival for the groups did not significantly differ.

2. Splenectomy (VII, 7XX, EA100)

The role of splenectomy on the natural history of chronic granulocytic leukemia is being investigated. Thus far, 21 patients have had a prophylactic splenectomy. Nine patients have entered blastic transformation and eight of these received chemotherapy. The median survival of this latter group is 6 months (range 3-10), despite absence of complete remission. The general supportive care of such patients was felt to have been markedly improved. There was only one complication of the splenectomy - postoperative bleeding in a single case.

3. Treatment of Blastic Phase of Chronic Granulocytic Leukemia (VI, 6X1, LA100)

Since the demonstration that vincristine and prednisone were capable of inducing hematologic remission in 30% of a series of 30 consecutive patients with blastic crisis of CGL, a randomized trial of this combination versus arabinosyl cytosine (ARA-C) (200 mg/m^2) and 6-Thioguanine (200 mg/m^2) was begun. To date 20 patients have been studied. Eight have received ARA-C/6TG with one complete and 2 good partial responses. Twelve have received vincristine, prednisone with one complete and four partial responses. Of interest is the observation that the one complete remitter in the latter group presented with hypodiploidy (44 Ph'). Including cases from our previous study 5/6 patients who presented with hypodiploidy at time of blastic crisis achieved a complete hematologic remission. We have a further patient treated in consultation at D.C. General Hospital who survived 36 months after complete remission with VCR/Pred. she also was hypodiploid. This makes a total of 6/7 cases who

achieved remission with only VCR/Pred. Crossover to opposite drug regimen was attempted in 10 cases with a partial response in one case.

C. Laboratory Studies

1. Effect of Humoral Substances on the Kinetics and Maturation of Stem Cells (VI, 6X2, SA50, EA50)

The in vitro culture technique has been used extensively to evaluate the functional status of marrow cells in man and experimental animals. We have used this technique to study (a) the effect of anabolic steroids on the growth of granulocytic precursor cells with special emphasis on the cell cycle of granulocytic precursor cells. The administration of testosterone salts and the pure base both have resulted in increased colony growth in vitro. In addition, H_3TdR labelling and DNA incorporation studies demonstrated an enhanced rate of DNA synthesis. This was evaluated functionally by the demonstration that ARA-C and hydroxyurea kill a higher fraction of CFU-C in testosterone treated animals. This was also demonstrated to be the case in vitro. Collaborative efforts with Dr. Alan Rosenblum resulted in the confirmation that testosterone in vitro will increase the CFU-C of normal human marrow cells.

2. Effect of Conditioned Medium on the Maturation of Malignant "Stem" Cells (VI, 6XL, SA50, EA50)

The potential of malignant cells to be induced to mature in vitro with addition of humoral factors was studied in the FRD-leukemia (murine viral induced).

The addition of conditioned medium from mouse L-cells in culture prompts a brisk growth of leukemic CFU-C but by 12 days of culture the number of colonies with or without conditioning becomes equal, suggesting that the conditioned medium prompts a more rapid proliferation. However, examination of the colonies produced revealed that on the 12th day of culture the composition of the 2 different cultures was radically different. Cultures of leukemic cells (in the presence of conditioned medium) yielded 42% immature cells with 58% recognizable mature granulocytes and macrophages, while the cultures in the absence of conditioned medium showed 97% immature leukemic cells despite and equal number and size of colonies per plate. This suggests that some factors may induce maturation of malignant cells in vitro without altering the growth kinetics of cells.

Reculture of the cells derived from 12 day old plates revealed an abundant and rapid growth of those cells derived from the "condition-less" medium. Thus there appears to be an adaptation by the malignant cells to the absence of conditioned medium such that repeat culture in the presence of L-cell medium results in a marked proliferation.

3. Effect of In Vitro Culture on Human Chronic Granulocytic Leukemic Cells: Leukocyte Alkaline Phosphatase (LAP) in Cells Derived from Cultured Stem Cells (VI, 6X2, SA50, EA50)

CGL cells from marrow and peripheral blood have been cultured in methylcellulose medium (HEK-conditioned medium). The leukocyte alkaline phosphatase (histochemical) was determined in cell suspensions derived from 0, 7, 10, 16 day cultures. By 10 days a significant increase in the numbers of LAP + cells was noted approaching normal. These findings suggest that the LAP activity of CGL granulocytes is subject to change with conditions of growth and further illustrates that the activity may be suppressed in vivo by the extent of granulocytic hyperplasia.

4. Studies of In Vitro Granulocyte Production of Patients with Hodgkin's and Non-Hodgkin's Lymphomas (VI, 6X1, LA50, SA50)

To ascess the acute and chronic effects of tumor and chemotherapy on bone marrow function, the in vitro granulocyte colony formation (CFU-c) was studied in patients with advanced lymphoma before and after treatment. Using a semi-solid methylcellulose system the CFU-c of 42 normal volunteers was 2.98(0.9 to 6.25). The mean CFU-c at diagnosis of 19 patients with advanced Hodkgin's disease was low, 1.22(0.00 to 2.4), differing from control values ($p<.005$). The CFU-c of patients with documented marrow involvement did not differ from advanced stage patients without proven marrow disease. In non-Hodgkin's lymphomas, patients with diffuse histiocytic and lymphocytic histologies had lower CFU-c's than those with nodular patterns. The mean CFU-c of 15 patients with diffuse lymphoma was 1.04 (0.00 to 2.00), and differed from controls ($p<.005$), whereas the mean CFU-c of 15 patients with nodular lymphoma did not differ from controls (mean 2.15, range 0.38 to 6.10). The cellular composition of the tumor (histiocytic, lymphocytic, or mixed) did not relate to CFU-c. Two patients studied serially prior to each of 6 cycles of MOPP therapy had low initial CFU-c's. CFU-c's entered the normal range following the first cycle of chemotherapy and remained normal during subsequent courses. Three of 4 patients in remission and off MOPP therapy for up to 6 years had normal CFU-c's. Patients with advanced Hodgkin's disease and diffuse histiocytic and lymphocytic lymphoma have impaired CFU-c's prior to treatment, whereas those with nodular lymphomas have normal CFU-c's. The low CFU-c may reflect non-specific inhibitory effect related to the biologic aggressiveness or volume of tumor. The low colony formation returns to and remains normal following successful drug control of the tumor.

5. Investigation of Usefulness of Nitroblue Tetrazolium (NBT) test in Determining Fever Source in Patients with Lymphomas (VI, 6X2, EA100)

A prospective study of NBT in normal and lymphoma patients has determined that the two populations are equal in reactivity. Lymphoma patients with

fever as a constitutional sign of underlying disease have normal NBT score whereas bacterially infected patients have a positive test. Thus, the NBT serves as a useful adjunct in determining the source of fever in lymphoma patients. Chemotherapy including prednisone does not effect the reactivity of white blood cells to phagocytosis and NBT dye reduction. Procarbazine may cause a false positive test by generation of H_2O_2 during its metabolism.

5. Studies of Leukocyte NADH Oxidase (VI, 6X2, SA50, EA50)

Neutophil NADH oxidase has been suggested as the primary enzyme involved in bacteriocidal activity, and deficient in patients with chronic granulomatous disease who are predisposed to infection. A sensitive micro assay system has been developed for the measurement of NADH oxidase in order to determine the effect of disease and chemotherapy on the enzyme's activity.

Honors and Awards:

Dr. Anderson

1. "The Usefulness of the Nitroblue Tetrazalium Test (NBT) in Determing Fever Source in Lymphoma." Presented at American Society of Clinical Oncology Annual Meeting, April 1973.

Dr. Canellos

1. "Chronic Myelogenous Leukemia." Presented at University of North Carolina, Chapel Hill, N.C., June 1972.

2. "Acute Leukemia." Presented as Lecture Washington Hospital Center, December, 1972.

3. "Current Concepts in Chronic Leukemia." Presented at Georgetown University School of Medicine, February 1973.

4. "Current Therapeutic Concepts." Presented at Chronic Leukemia 7th National Cancer Conference, Los Angeles, California, September 1972.

5. "Hypercalcemia in Leukemia Lymphoma." Presented at Academ. of Sciences Symposium, March 1973.

6. "Dibromomannitol: A randomized comparison with busulfan in the treatment of chronic granulocytic leukemia." Presented at American Society of Clinical Oncology Annual Meeting, Atlantic City, New Jersey, April 1973.

7. "Effect of Androgens in Granulopoiesis in vitro and in vivo." Presented at American Federation of Clinical Research, Atlantic City, April 1973.

8. "The Myeloproliferative Disorders." Lecture, Course in Laboratory Medicine, Georgetown University School of Medicine, January 1973.

9. "Management of Malignant Hematology Tumors." Presented at Hematology Grand Rounds, Peter Bent Brigham Hospital, Boston, Massachusetts, December 1972.

Dr. DeVita

1. "Chronic Granulocytic Leukemia." Lecture to the Division of Hematology, Stanford University School of Medicine, July 1972.

Publications:

1. Canellos, G.P., Whang-Peng, J., Schnipper, L., Brown, C.H.: Prolonged cytogenetic and hematologic remission of blastic transformation in CGL. Cancer 30: 288, 1972.

2. Canellos, G.P., Nordland, J., and Carbone, P.P.: Splenectomy for thrombocytopenia in CGL. Cancer 29: 660, 1972.

3. Canellos, G.P. and Whang-Peng, J.: The Philadelphia Chromosome Preleukemic State. Lancet 2: 1227, 1972.

4. Canellos, G.P.: Hypercalcemia in leukemia. Ann. N.Y. Acad Sci., 1973. (In press)

5. Canellos, G.P., DeVita, V.T., Young, R.C., Chabner, B.A., and Schein, P.: Current therapeutic aspects of chronic granulocytic leukemia. Proceedings of 7th National Cancer Conference, 1972.

6. Rosenoff, S.H., Canellos, G.P., O'Connell, M., Wiernick, P.: Mediastinal adenopathy in granulocytic leukemia. Arch Int. Med. (In press)

Serial No. NCI-3420
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Kinetics of Cellular Proliferation

Previous Serial Number: Same

Principle Investigators: Robert C. Young, Vincent T. DeVita, Philip S. Schein, Bruce A. Chabner, Stephen H. Rosenoff

Other Investigators: Charles E. Myers, Vincent T. Oliverio

Cooperating Units: Laboratory of Chemical Pharmacology, NCI

Man Years:

Total:	3.5
Professional:	2.25
Other:	1.5

Project Description:

The goals of the tumor cell kinetic program of the Medicine Branch over the past 5 years have been:

1. To examine and develop methodology that might be useful in obtaining cell kinetics information in individual patients in a time period that may allow application of these data to the patient's therapy.

2. To describe the kinetics of human tumors, compare them to animal models, and to compare results to normal tissues, to the same tumor in different patients, and to different metastatic sites of the same tumor in the same patient.

3. To examine the manner in which cell cycle characteristics are changed in response to exposure to drugs.

4. To approach the problem of manipulating tumor growth characteristics in such a way as to increase its susceptibility to chemotherapeutic agents.

A. Animal Studies

1. Cell cycle effects of the nitrosoureas (IIB, IV 4 x 2, 2B2 LA 100)

Continued interest in the unusual kinetic affects of the nitrosoureas has been the source of a series of studies using both BCNU and more recently MeCCNU. Cell cycle studies with BCNU were completed and published in the past year. They revealed that transplant generations exposed previously to BCNU have altered cycle times suggesting survival and selection of a subline of L1210 cells with longer cycle times.

Kinetic studies with MeCCNU have demonstrated a prolongation of the S phase occurs with doses as low as 4 mg/kg; no persistent alteration in growth fraction is produced by MeCCNU; the generation time of L1210 cells regrowing after MeCCNU is slightly prolonged compared to normal and would seem to result from a selection out of cells with shorter cell cycle times. A new technique for simultaneously studying the effect of chemotherapeutic agents on normal and tumor tissues has been developed and applied to the administration of MeCCNU in vivo. Simultaneous studies on ascites tumor, bone marrow and gastrointestinal mucosa have shown a differential effect on the suppression of DNA synthesis in tumor and normal tissues and this differential recovery can be used to select subsequent dose schedules and enhance tumor response to MeCCNU. Schedules designated in this manner can be shown to double the cure rate in mice bearing L1210 leukemia. Studies are presently under way testing MeCCNU in a solid tumor system utilizing B16 melanoma.

2. Methotrexate (IIB, IV, 4 x 2, 2B2 LA100)

Studies are underway in cooperation with the Laboratory of Chemical Pharmacology assessing the effect of multiple doses of Mtx on the suppression and recovery of DNA synthesis in tumor tissue and in normal target tissues in vivo. These kinetic data have been correlated with Mtx blood levels. The threshold level for DNA suppression by Mtx is $1\text{-}2 \times 10^{-8}$M. This emphasizes the importance of the low persistent levels of Mtx in contributing to the toxicity of this agent. Work is in progress to determine the mechanism of leukovorin rescue by following the kinetic alterations in DNA, RNA and protein synthesis after Mtx and after rescue with leukovorin. Studies have also been completed on the effect of carboxypeptidase on DNA synthesis in CDF mice bearing L1210 ascites tumor and indicate that DNA synthesis in the tumor may be suppressed while normal marrow DNA synthesis is unimpaired.

3. Cyclophosphamide, BCNU, and Cytosine Arabinoside (IIB, IV, 4 x 2, 2B2, LA100)

Studies on the suppression and recovery of DNA synthesis in normal and tumor tissues have been completed and demonstrate that following a single dose of cytoxan or BCNU, a pattern of suppression and recovery of DNA synthesis has been observed which was different in normal host tissues from that seen in the L1210 ascites. These patterns were predictive of the toxicity produced by subsequent doses of chemotherapy and also could be used to enhance therapeutic effect in a tumor bearing animal.

4. 5-Fluorouracil (IIR, IV, 4 x 2 2B2, LA100)

Similar studies have been completed in GP mice bearing Erlich's ascites tumor and in CDF_1 mice bearing P1534 ascites tumor. For a sensitive tumor system such as P-1534 a schedule dependent improvement of survival is possible by schedules designed around kinetic recovery patterns. Cooperative studies with the Laboratory of Chemical Pharmacology have developed an assay of the tissue levels of the active principle (5-FdUMP) using an extremely sensitive enzymatic assay based on the specific suppression of Thymidylate synthetase produced by 5-FU.

B. Human Studies (IIB, IV, 4 x 2, 2B2, LA100)

Mtx studies on the effect of Mtx on the suppression and recovery of DNA synthesis in human bone marrow and human ovarian carcinoma ascites cells have been completed in three patients with a simultaneous study of blood and ascites levels of Mtx. As with animals, DNA synthesis in humans in vivo seems to recover when the concentration of Mtx in the blood falls to 1×10^{-8} M or lower and that the kinetic suppression and recovery can be predicted from a knowledge of the patterns blood levels of the agent. In these three patients all of whom failed to respond to Mtx, the ovarian ascites tumor recovered at concentration of Mtx and one log higher than did the normal bone marrow.

When the B_{16}-Melanoma-MeCCNU model is completed, we plan to use a similar system in man with simultaneous studies on bone marrow and melanoma nodules, to see if schedules of MeCCNU can be derived by studying differential DNA synthesis recovery patterns for the human marrow and the melanoma nodules.

Honors and Awards:

Dr. Young

1. "Kinetic Alterations Induced by Chemotherapeutic Agents In vivo," presented at International Symposium on the Eradication of the Last Neoplastic Cell, Rio de Janiero, July, 1972.

2. "Cell Kinetics and Chemotherapy," presented at Pathologic Anatomy Branch Conference, NCI, December, 1972.

3. "Kinetic Alterations Induced by ARA-C, Mtx and 5-FU in Normal and Tumorous Target Tissues," presented at Department of Hematology and Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri, March, 1973.

4. Myers, C.E., Oliverio, V.T., and Young, R.C.: Kinetic Alterations Induced by 5-FU In Vivo: The Simultaneous Effect of Chemotherapy on Bone Marrow, Intestinal Mucosa and Erlichs Ascites Tumor. Presented

at American Association for Cancer Research Annual Meeting, Atlantic City, N.J., April, 1973.

5. Young, R.C., and Goldberg, G.: The Effect of Methyl CCNU on the Cellular Kinetics of Normal and Leukemic Tissues In vivo. Presented at American Association for Cancer Research Annual Meeting, April, 1973.

6. Young, R.C. and Chabner, B.A.: An In vivo Method for Monitoring Differential Effects of Chemotherapy on Target Tissues in Animals and Man: Correlation with Plasma Pharmacokinetics. Presented at American Society for Clinical Investigation Annual Meeting, April, 1973.

Dr. DeVita

1. "Cell Kinetics," Lecture to Department of Medicine, Stanford University School of Medicine, July, 1972.

Publications:

1. Young, R.C.: The effect of methyl CCNU (NSC 95441) on the cellular kinetics of normal and leukemic murine tissues in vivo. Cell and Tissue Kinetics 6:35, 1973.

2. Young, R.C., Goldberg, D., and Schein, P.S.: Enhanced antitumor effect of cytosine arabinoside given in a schedule dictated by in vivo kinetic studies. Biochemical Pharm. 22: 277, 1973.

3. Chabner, B.A., and Young, R.C.: Threshold methotrexate concentration for in vivo inhibition of DNA synthesis in normal and tumorous target tissues. J. Clin. Invest. (In press)

4. DeVita, V.T., Bray, D.A., Bostick, F., and Bagley, C.M.: The effect of chemotherapy on the growth of leukemia L1210: II Persistance of a nitrosourea induced change in the growth characteristics of transplant generations. Cell and Tissue Kinetics 5: 459-466, 1972.

Serial No. NCI-6500
1. Medicine Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physiologic Disposition, Metabolism, Excretion and Cellular Interaction of Antitumor Agents

Previous Serial No.: Same

Principle Investigators: Philip Schein, Vincent T. DeVita, Bruce Chabner, Robert Young, James Arseneau, George Canellos, Robert Sponzo

Other Investigators: Vincent Oliverio, David Johns, David Cooney

Cooperating Units: Laboratory of Chemical Pharmacology, Laboratory of Toxicology

Man Years:

Total:	2.75
Professional:	1.50
Other:	1.25

Project Description:

A significant area of interest in medical oncology is understanding the pharmacokinetics and drug-cell interaction of established and newer antitumor agents. The Medicine Branch has conducted pharmacokinetic studies on new agents under actual treatment circumstances while drugs are undergoing phase I evaluation. Also, studies have been performed on established but pharmacologically poorly understood antitumor agents, such as cyclophosphamide.

A. Clinical Pharmacology

1. The Nitrosoureas (VI, 4X2, LA100)

The physiologic disposition of radiolabelled CCNU and MeCCNU was studied in patients receiving these drugs as part of their early clinical trial. CCNU was given orally in doses of 30-100 mg/m^2 and MeCCNU in doses of 120-290 mg/m^2. With both drugs significant plasma levels of radioactivity were detected in as early as 10 minutes with peak levels occurring at 1 to 6 hours. Plasma levels of isotope were prolonged and the patterns of decline of radioactivity from plasma were similar for the same labelled moieties of CCNU and MeCCNU. Approximately 60% of the radioactivity given was excreted in the urine within 48 hours and of this more than 50% was excreted in the first 12 hours. Radioactivity was found in the cerebrospinal fluid of patients given both drugs and

with CCNU these levels paralleled concurrent plasma levels. The degradation of both drugs appears to be extremely rapid since the intact drug could not be detected in any plasma, CSF, or urine sample. These studies suggest a somewhat erratic absorption pattern for methyl CCNU and as with the other nitrosoureas studies in the past, antitumor activity related to one of the degradation products of the drug.

2. Cyclophosphamide (VI, 6X2, LA100)

Cyclophosphamide (CTX) is one of the few antitumor agents requiring microsomal oxidative conversion to its active form. Few clinical studies are available examining the rates of conversion of CTX to its alkylating metabolites in relation to the distribution, degradation, and excretion of the intact drug and metabolites. Utilizing both radiolabelled CTX and the NBP assay for alkylating activity these studies were completed in the past year. The human pharmacology of cyclophosphamide was investigated in 26 patients who received cyclophosphamide ^{14}C in doses of 6 to 80 mg/kg i.v. Levels of the intact drug in plasma and urine and excretion of ^{14}C label in breath and stools were determined by liquid scintillation counting. Plasma and urine alkylating activity was measured by reaction with 4-(4-nitrobenzyl)pyridine. Protein binding of cyclophosphamide and plasma alkylating metabolites were determined by plasma ultrafiltration.

Injected cyclophosphamide distributed rapidly into 64% of body weight and plasma cyclophosphamide half-life in patients without prior drug exposure was 6.5 hr. Not more than 20% of injected cyclophosphamide was excreted intact in urine at any dose level.

Plasma alkylating metabolites were 56% bound to plasma proteins. After a 40-mg/kg, peak unbound alkylating activity averaged 13.3 mμmoles/ml, and in most patients at this dose alkylating activity in the plasma was measurable for 24 hr. Sixty-eight percent of injected ^{14}C label was excreted in urine. Breath and fecal excretion were negligible.

In a regimen of five consecutive daily cyclophosphamide administrations, cyclophosphamide half-life was shorter and peak alkylating levels were constantly higher on the 5th day than on the 1st day. Prior patient treatment with allopurinol resulted in significantly longer cyclophosphamide half-life, but concomitant prednisolone treatment had no effect. The effect of hepatic metastases on cyclophosphamide metabolism was unclear. Moderate renal failure in one patient resulted in prolonged retention of alkylating materials in plasma and severe toxicity.

Although patients with and without prior exposure to microsomal enzyme-inducing drugs demonstrated marked variation in plasma cyclophosphamide half-life and peak alkylating levels, the total concentration X time (CXT) product remained relatively constant for a given cyclophosphamide dose, suggesting that alterations in the rate of cyclophosphamide metabolism by drugs or liver metastases in the absence of renal failure will not change toxicity or therapeutic effect.

3. Streptozotocin (IV, 6X2, LA100)

Studies of the pharmacologic distribution and excretion of streptozotocin have been carried out in seven patients and mice and dogs using a spectrophotometric assay.

The drug was administered intravenously to patients in doses ranging from 200 mg/m^2 to 1,500 mg/m^2. The plasma half-life was 15 minutes at all doses, with no detectable drug by 2 hours after administration. Ten to 20 percent of each total dose is excreted in the urine with the N-nitroso group intact by 2 hours after infusion.

Drug distribution studies in mice have demonstrated concentration of streptozotocin in liver and kidney. The drug doses not pass the blood brain-barrier with the N-nitroso group intact.

Studies are in progress using ^{14}C-methyl labelled streptozotocin and 1-methyl-1-nitrosourea to determine whether the differences in the pharmacology of these two L-1210 active agents (diabetogenicity, lack of myelosuppression, passage of the blood brain barrier can be explained by the presence of the glucose portion of the streptozotocin molecule.

4. Gallium Citrate (IV, 4X2, LA100)

Studies of the physiologic distribution of ^{67}Ga citrate in patients with chronic granulocytic leukemia have shown accumulation of the nuclide in circulating and extramedullary soft tissue collections of neoplastic cells. The intracellular binding site of ^{67}Ga is being investigated and appears likely to be related to lysosomes or lysosomal-like proteins. The propensity of ^{67}Ga to bind to granulocytes, perhaps, because of their rich lysosomal content, makes this nuclide useful both from a clinical and experimental standpoint. In addition to using scintiscanning techniques to localize collections of granulocytes, the nuclide may provide a useful and fairly specific label in the study of granulocytic kinetics.

A further important clinical use of ^{67}Ga citrate may be in scanning to localize inflammatory lesions. The kinetics of ^{67}Ga incorporation into experimental inflammatory lesions have been studies in monkeys with Dr. Louis Gelrud of the Department of Nuclear Medicine, CC, NIH and the incorporation has been shown to correspond to the granulocyte mass at the inflammatory site. This phenomenon may provide a useful non-invasive method of localizing inflammatory lesions, especially in such difficult clinical diagnostic problems as occult thrombophlebitis or sub-diaphragmatic abcess.

In vitro studies of the relation of gallium blinding by various animal tumor systems to the lysomal content of these tumors are in progress, in an attempt to establish lysosomal binding as the chief mechanism to account for the ^{67}Ga concentration in certain tissues.

5. Carboxypeptidase G_1 (III, 3X2)

Progress has been made in the large scale production and purification of this antitumor agent, in conjunction with the New England Enzyme Center, Tufts University. A one-step purification to homogeneity has been accomplished using a specific elution with glutamate from carboxymethyl-cellulose. Clinical trials with this agent are being planned in New Haven by Dr. Joseph Bertino.

6. Procarbazine (VI, 6X2, SA50, EA50)

A study has been completed to determine the extent the host drug metabolism can modify procarbazine such that its potent in vitro oxidation hemolytic potential is ameliorated. A series of in vitro experiments have clearly demonstrated that procarbazine is capable of oxidizing reduced glutathione. This in turn contributes to rapid disease in red cell sulfhydryl (-SH) groups. There is a secondary oxidation of oxyhemoglobin to form methemoglobin with red cell membrane damage and increased osmotic fragility. We demonstrated that this is effected by the ability of procarbazine to produce H_2O_2 in situ. Glucose-6-phosphate dehydrogenase (G-6-PD) deficient red cells are more sensitive then normal. Larger doses of parenteral procarbazine were administered to patients who received a cohart of chromium labelled G6PD deficient red cells (ABO Rh compatible). No alteration of survival was noted. Furthermore, a series of procarbazine metabolites were examined for their in vitro oxidative potential. The hydrazone which is represents a minor metabolite was active, however, the major metabolites were totally inactive suggesting that rapid in vivo conversion of the drug occurs to metabolites which lack erythrotoxic activity.

7. Cytosine Arabinoside (IV, 4X2, LA100)

Cytosine arabinoside resistance in human leukemia has been ascribed to increased levels of the catabolic enzyme, cytadine deaminase. We have recently begun a study of the biochemical properties of this enzyme, as isolated from normal and leukemic myelocytic cells. Initial results of this study have been reported at the American Association for Cancer Research, including data on the molecular weight, purification, kinetic properties, and substrate specificity. Attempts will be made in the coming year to purify this enzyme to homogeniety and to determine the regulation of deaminase activity in human leukemic cells.

8. 5-Azacytidine (IV, 4X2, LA100)

We have also investigated the metabolism of 5-azacytidine by leukemic cells, and have shown that cytidine deaminase will inactivate this agent, although the affinity of deaminase for 5-azacytidine is four-fold less than the affinity for ara-C. This finding raises the possibility of cross-resistance developing to these two agents on the basis of increased deaminase levels in leukemic cells.

B. Mechanism of Action of Antitumor Agents

1. Streptozotocin (IIA, 2AX, LA100)

In previous studies it had been demonstrated that the diabetogenic, but not the antileukemic, activity of streptozotocin could be correlated with depression of pyridine nucleotides, NAD and NADH.

Recent investigations performed in collaboration with Dr. David Cooney, in the Laboratory of Toxicology, on isolated islets of Langerhans of the mouse have confirmed the ability of streptozotocin to reduce NAD concentrations in this tissue, and the use of nicotinamide to prevent this diabetogenic activity. Employing ^{14}C-nicotinamide as a precursor of NAD, a decrease in NAD synthesis in pancreatic islets and livers was demonstrated. This could be correlated with a decreased uptake of ^{14}C-nicotinamide and a block in incorporation of the label into nicotinamide mononucleotide.

C. Drug-Cell Interactions

1. Identification of Non-Myelosuppressive Nitrosourea Antitumor Agents (IIA, 2AX, LA100)

Structure-activity studies with streptozotocin and 1-methyl-1-nitrosourea have suggested that the absence of myelosuppression of the former compound was related to the glucose carrier. Studies were undertaken to examine the ability of glucose carriers to modify the bone marrow toxicity of other cytotoxic moieties. The first compound to be tested is 3-(tetraacetyl glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea, GCNU, synthesized by Dr. John Montgomery of the Southern Research Institute. GCNU is active against the L-1210 leukemia, ILS of 80-140%, without bone marrow toxicity at an LD_{10} dose of 15 mg/kg. Despite its structural similarities to streptozotocin, GCNU is not diabetogenic and does not depress NAD concentration.

Similar studies are now being undertaken with three additional glucose containing nitrosourea antitumor agents.

D. Prediction of Clinical Drug Toxicity from Animal Data (III, 3X1, LA100)

The section has been concerned with the ability of animal toxicologic studies to predict qualitative and quantitative toxicities encountered during clinical study of anticancer agents. Correlations between animal and human data have been made to allow physicians to better interpret toxicologic data.

Honors and Awards:

Dr. Schein

1. "Qualitative Aspects of Drug Toxicity in Prediction from Laboratory Animals to Man." Presented at Fifth International Congress on Pharmacology, San Francisco, July 1972.

2. "Streptozotocin - Mechanism of Action and Clinical Pharmacology." Presented at the Johns Hopkins Oncology Division Conference, February 1973.

3. "GCNU, A Non-myelosuppressive Antitumor Agent." Presented at American Association for Cancer Research Annual Meeting, April 1973.

4. "Prediction of Drug Toxicity from Laboratory Animals to Man," and Current Pharmacologic Concepts in Cancer Chemotherapy." Presented at Clinical Pharmacology Lecture Series, Philadelphia General Hospital, May 1972.

5. "Clinical Pharmacology of Streptozotocin." Presented at Baltimore Cancer Research Center, January 1973.

6. "Clinical Pharmacology of Streptozotocin." Presented at Children's Cancer Research Center, Boston, Massachusetts, December 1972.

Dr. DeVita

1. "Clinical Pharmacology of Cyclophosphamide." Lecture to Department of Oncology, Stanford University, School of Medicine, July 1972.

Dr. Chabner

1. "The Clinical Pharmacology of Folate Antagonists." Presented at Letterman General Hospital, San Francisco, California, July 1972.

2. "Carboxypeptidase G-1, A Folating Cleaving Enzyme." Presented at University of North Carolina, October 1972.

3. "Studies of Carboxypeptidase G-1, A Folate Cleaving Enzyme." Presented at Children's Cancer Research Center, Boston, Massachusetts, Dec. 1972.

4. "Clinical Pharmacology of Methotrexate." Presented at Yale University Medicine School, February 1973.

5. "Threshold Plasma Concentration for Inhibition of DNA Synthesis by Methotrexate." Presented at International Congress of Pharmacology, San Francisco, California, July 1972.

6. "Isolation and Characterization of Human Leukemia Cytidine Diaminase." Presented at American Association for Cancer Research, Atlantic City, April 1973.

Publications:

1. Schein, P. S., Alberti, K. G. M. M., Williamson, D. H.: Effects of streptozotocin on carbohydrate and lipid metabolism in the rat. Endocrinology 89: 197-204, 1972.

2. Rakieten, H., Gordon, B. S., Beaty, A., Cooney, D. A., Davis, R. D., Schein, P. S.: Pancreatic islet cell tumors produced by the combined action of streptozotocin and nicotinamide. Proc. Soc. Exp. Biol. Med. 137: 280-283, 1971.

3. Henry, M. C., and Schein, P. S.: Beagle dog as predictor of schedule-dependent toxicity of 5-fluoro-2-deoxyuridine. Toxicol. App. Pharmacol. 23: 532-534, 1972.

4. Schein, P. S., Davis, R. D., Cooney, D. A.: Qualitative aspects of drug toxicity in prediction from laboratory animals to man. Proc. 5th Int. Cong. Pharm. (In press)

5. Schein, P. S., Anderson, T.: The efficacy of animal studies in predicting clinical toxicity of cancer chemotherapeutic drugs. Int. J. Clin. Pharm. (In press)

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1. Office of the Chief, Pediatric Oncology Branch
2. Uganda Cancer Institute Makerere University, Kampala, Uganda

PHS-NIH
Individual Progress Report
July 1, 1972 through June 30, 1973

Project Title: Study of Burkitt's lymphoma, Hodgkins disease, malignant melanoma, hepatocellular carcinoma and Kaposi's sarcoma in Ugandan Africans

Previous Serial Number: NCI-3422

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Other Investigators: Charles L. M. Olweny, Sebastian K. Kyalwazi; Dhoval S. Dhru, Thomas Toya, Ian T. Magrath, Ian W. C. McAdam

Cooperating Units: Pathologic-Anatomy Branch, NCI; Immunology Branch, NCI, Departments of Surgery, Pathology, Medicine and Pediatrics, Makerere University, Kampala, Uganda

Man Years:

Total:	15
Professional:	5
Other:	5

Project Description: Linear Array Codes-IV 4X1, IV 4X2, V 5XX, VI 6X1, and VII 7XX.

Introduction:

The Pediatric Oncology Branch, NCI, supports a contract (PH 43-67-1343) with Makerere University in Kampala, The Uganda Cancer Institute. This Institute is divided into two separate clinical wards, the Lymphoma Treatment Center for childhood tumors and Burkitt's lymphoma, and the Solid Tumor Center, devoted to study of hepatocellular carcinoma, malignant melanoma, and Kaposi's sarcoma in adults. Due to political disturbances in Uganda in the Fall of 1972, the NCI advisory staff withdrew from Uganda, and the clinical activities were reorganized. Dr. Ian Magrath, a British Cancer Campaign Oncology Fellow, maintained all clinical and investigative activities at the Lymphoma Treatment Center until the arrival of Dr. Charles Olweny in March, 1973. Dr. Olweny is a Ugandan who received 2 years of

Oncology training at NCI. The continuity of The Burkitt lymphoma, Hodgkins disease and childhood solid tumor studies has thus been maintained both with respect to clinical protocols and patient followup. Clinical activities at the Solid Tumor Center have been restricted to clinical trials in patients with Kaposi's sarcoma under the leadership of Professor Kyalwazi.

I. Lymphoma Treatment Center (LTC)

In the past year, 82 children with malignant disease were admitted to the Lymphoma Treatment Center. A further 25 patients were referred with a possible diagnosis of malignancy were found to have non-malignant conditions. One adult with chronic lymphatic leukaemia is under treatment, and 8 patients with tropical splenomagaly syndrome were investigated. The diagnoses in these patients were as follows:-

Burkitts lymphoma	46
Lymphoblastic lymphoma	8
Stem cell /Histiocytic lymphoma	7
Hodgkins disease	3
Lymphoma of unknown type	2
Embryonal sarcomata	4
Abdominal sarcoma	1
Neuroblastoma	4
Retinoblastoma	1
Kaposi sarcoma	1
Wilm's tumour	3
Acute lymphoblastic leukaemia	1
Chloroma (AML)	1
Tuberculosis	4
Fibous dysplasia	2
Chromic lymphatic leukaemia	1
Dermoid cyst	2
Idiopathic splenomagaly	8
Inflammatory conditions	15
Miscellaneous	2
Total	116

The major research interest has continued to be Burkitt's lymphoma, but chemotherapeutic protocols for other types of lymphoma and childhood sarcomas are under study.

Staging and Survival. The large amount of data which has accumulated over the 6 years since the establishment of the LTC has prompted a review of the

staging system in Burkitt's lymphoma. One hundred and ten evaluable patients have been analysed in respect of prognosis related to tumour site. This analyses has led to a revision of the original staging classification of Burkitt's lymphoma, which more accurately reflects both the risk of relapse and mortality. This staging scheme is summarized below:

Revised Staging of Burkitt's lymphoma

Previous Staging		Revised Staging
Stage I	Single facial tumor	Solitary extra-abdominal tumor
Stage IA	- - -	Resected intraabdominal tumor
Stage II	Two or more facial tumors	Multiple extra-abdominal tumors
Stage III	Visceral and/or osseous tumors	Intra-abdominal tumor with or without facial tumors
Stage IV	CNS (malignant cells in the CSF) or generalized bone marrow involvement	Intra-abdominal tumor (Stage III) with sites of tumor other than facial

The essential changes in the revision are: 1) the recognition of an improved prognosis in patients without intra-abdominal involvement regardless of site; 2) the realization that CNS involvement does not carry a uniformly poor prognosis; and 3) the marked improvement is survival in patients who have resected (>90%)abdominal tumors. This revision reflects several important therapeutic features including improved management of meningeal involvement (>50% long term survivors), the role of reductive surgery in patients with resectable or partially resectable abdominal tumors, and the importance of early recognition and prompt treatment of relapse. A summary of relapse and mortality using the revised staging scheme appears below:

Patients Analyzed

	No.
Patients admitted prior to January, 1972	137
Early death	18
Partial or no response	9
Total evaluable	110

Stage (Revised)

	I	IA	II	III	IV
No.	25	8	27	28	22
Dead	6	0	4	12	13
Relapse	8	2	17	22	17
No relapse	17	6	10	6	5

Thus mortality for Stage I and IA is about 18% and for Stage II is 15%; the only reason for separating these groups is that the risk of relapse is only 30% for Stage I and IA but 63% for Stage II patients. Mortality in Stages III and IV is 42% and 60% respectively, with the risk of relapse is 77% and 78% respectively. The revised staging scheme will be used to analyze restrospectively the data from all controlled clinical trials since 1967 to acertain whether new and useful treatment information will be available. In addition, clinical, immunologic and serologic data are under review to discern any correlations with the new staging system.

The prognostic importance of the site of the first relapse (same, new, or CNS alone) has been investigated in 175 patients by comparing survival rates in these three groups. There is no significant difference. If the duration of remission is taken into account, however, it is clear that patients who relapse within 13 weeks with tumour at a previously uninvolved site (usually CNS) have a significantly worse prognosis that patients relapsing with new site involvement after 26 weeks, or with recurrence at the same site within 13 weeks. Remission duration prior to same site, or CNS alone relapse does not appear to be of inportance in terms of survival, but most CNS relapses occur within 13 weeks. Patients who relapse with CNS involvement and tumour at other sites have a significantly worse prognosis than with other types of relapse patients ($P<.025$).

162 evaluable patients followed over the past 5 yrs have been analysed with respect to long term survival. Those who die within 2 weeks are not included, nor are patients in whom abdominal tumour was extensively resected (11 patients).

STAGE	No. Patients	Predicted survival at 100 weeks	Predicted survival at 200+ weeks
I	49	82%	82%
II	30	82%	82%
III	40	52%	52%
IV	43	29%	17%
	162		

Overall predicted survival at 200 wks and longer for all patients is 57%, and rises to 67% if patients with resected abdominal tumor are included. Only 2 of the 162 patients died after 100 wks (both were stage IV).

BCG Immunotherapy. In view of the failure of multiple doses of CTX, or or sequential therapy (TRIKE) to improve the overall survival compared to one or two doses of CTX, it was decided to attempt to non-specifically stimulate the patients' immune response with BCG, in order to possibly increase the host anti-tumour aggressive reaction. The trial was randomised and patients were stratified according to the prior staging systems. All previously untreated patients who achieved complete remission after two doses of CTX received 10 weeks of BCG scarification immunotherapy or no further treatment. Thirty-one patients are evaluable at the present time, and the results are shown in the following tables:

	No. Patients	Total Relapses (%)	Extra CNS Relapses (%)
BCG	17	9 (53%)	5 (29%)
Control	16	10 (63%)	9 (56%)

The apparent difference in extra CNS relapses is related to disproportionate representation of stages in the two groups. Thus when extra-CNS relapse is related to site of initial tumor, there appears an excess of patients with "revised" Stage IV disease amongst the controls. The greater number of CNS relapses in the BCG treated group also fits in with the excess of patients with "revised" Stage II in that group:

Relapse Frequency

	Total extra CNS relapses	Facial Tumour(I)	Abdomen alone(III)	Abdomen with CNS tumour(IV)
BCG	5	1/3	4/6	0/1
Control	9	0/2	5/9	4/4

There is no difference in the two groups with respect to relapse frequency or remission duration. It is as yet too early to assess survival differences.

It is of interest that in spite of this negative clinical result there was definite evidence in the BCG group that cell mediated immunity was stimulated. Thus in the BCG group cutaneous reactivity to recall antigens was increased by 43% as opposed to 3% in the control group. Reactivity to the Burkitt tumour cell lines, P_3J and Raji, was also increased in the BCG group (2 and 6 conversions respectively in 12 patients, as compared to 0 conversions in 5 patients in the control group).

Six control patients and 4 BCG patients were also tested with autologous tumour, but no conversions occurred. This would suggest that although general immunocompetence has been increased by BCG, specific anti-tumour reactivity aggression has not been altered, as measured by delayed hypersensitivity to autologous tumor extract. Further work is needed to elucidate the process of tumour sensitisation in Burkitt's lymphoma, and a new trial is being designed to combine BCG stimulation with tumor-associated antigen.

Prophylaxis of Meningeal Relapse with CCNU. Since meningeal Burkitt's lymphoma is a frequent form of relapse, an attempt to prevent this was made by administering 70 mg/M^2 CCNU to patients at the time of their second dose of CTX in a controlled randomized study. At present there are 19 patients in the control group and 20 in the CCNU treated group. The incidence of CNS relapse was identical in both groups, (5 patients in each) but there was a significant delay in the appearance of CNS tumour in the CCNU treated group($P = .008$ by Mann-Whitney U test). The value of such a delay is not clear, and further trials using multiple doses of CCNU may have been indicated. There is however, a signigicant excess of deaths in the CCNU treated group (10 compared to 2, $P<0.01$) which precludes further studies involving use of the drug at an early stage of the disease. Seven of the 10 patients died with tumour resistant to

chemotherapy, 2 had no tumour at post-mortem (one died of pneumonia, one from an idiosyncratic reaction to intrathecal methotrexate) and the last died at home following an episode of diarrhea and vomiting. The reason for this excessive mortality is not clear, and is not attributable to toxicity or staging disproportion.

Therapy of Meningeal tumor with Combined Intrathecal Treatment with ARA-C (day 1-3) and MTX (day 4) with or without Systemic Hydroxyurea (HU). It is too early to conclude this trial at the present time, since numbers are still small (11 in HU group, 9 in control). The preliminary data, summarized below, favors patients receiving HU as compared to controls:

	HU	No HU
No.	11	9
Presenting	3	3
Developing	8	6
Complete response	10	6
Relapse	2/10	3/6
Dead	3	6
Long survivors	5	2

Studies of Cell Mediated Immunity in Burkitt's Lymphoma. Serial immunological studies both in vivo and in vitro are undertaken on patients with the clinical course. There is now clear evidence that patients with Burkitt's lymphoma have impaired cell-mediated immunological reactivity at the time of presentation. The reactivity to 4 recall antigens is shown below:-

	No. Patients					Total
No. of positive reactions	0	1	2	3	4	
At admission	8	6	6	2	1	23
After at least a year of remission	0	3	7	4	4	18

More patients in the admission group have no reactivity or react to only one antigen (61%) than in the group with a long period of sustained remission (16%). These figures are statistically significant.

There is also a correlation between reactivity to recall antigens at presentation, and subsequent prognosis. Of 7 patients with complete anergia, who have been followed for at least 3 months, 4 have died whereas no patient has died in a group of 8 patients who reacted to 2 or more antigens at the time of admission.

In vitro lymphocyte reactivity to PHA correlates to some extent with cutaneous reactivity. In 8 patients with 2 or more skin positive tests at admission, only one had a stimulated : control ratio of below 90, whereas 4 of 9 patients did so in the anergic group. Serum from patients with a poor PHA response did not inhibit PHA reactivity of lymphocytes from a normal donor, excluding the possibility that lack of PHA responsiveness is due to a serum inhibitor.

Cutaneous reactivity to Raji and P_3J antigens at the time of presentation does not differ in frequency between patients with Burkitt's lymphoma and other types of malignancy:

	Patients tested	No. Positive Raji	No Positive P_3J
Burkitt's lymphoma	18	6	3
Other Malignancies	18	6	5

There does, however, appear to be a difference in the remission duration in Burkitt patients with positive reactions to Raji at presentation compared to those with no reaction. Although follow-up periods are still fairly short, only one patient with a positive response to Raji (and that was borderline) has relapsed. This patient had a negative reaction to Raji when re-tested a month after admission. In contrast, 8 of 11 patients unreactive to Raji on admission have subsequently relapsed.

This finding is of importance since it is possible that R aji cells carry an antigen common to all Burkitt cells. As such, this would be a very useful preparation to use for specific immunotherapy. The relationship of Raji reactivity to remission is also noticeable in patients who have been free from relapse for at least a year. At presentation only 2 of these particular patients were tested, and one was positive. After 6 months of remission, 7 were tested, with still one positive. When patients were tested after a year however, of 9 patients, 5 were positive. Four of these positive reactors had converted from a previously negative reaction at 6 months. It is also of interest that recall antigens tested at the same

time increased in reactivity. These findings are shown in the table below:

	Admission		6 Months		1 yr or more	
	Patients tested	+ves(%)	Patients tested	+ves	Patients tested	+ves
Raji	2	1	7	1	9	5
Recall	15	20(33%)	12	33(69%)	10	30(75%)

Thus it is possible that the increased reactivity to Raji reflects the increase in immunological reactivity to non-tumour antigens. It is interesting, however, that at 6 months, when recall antigen activity has been considerably improved, there is no increase in reactivity to Raji. In addition, the reactivity to P_3J does not increase with time (1 of 7 positive at both 6 months and 1 year).

Reactivity to autologous tumour has been observed in only 5 of 29 patients tested, although a further 5 patients had borderline results. In all cases reactivity occurred at a time when the patient was in remission. 3 of the 5 patients have relapsed subsequent to the +ve skin reaction, with subsequent conversion of reactivity.

To date lymphocytes from 7 patients at presentation or relapse, and from 10 patients with sustained remission for over 1 year have been studied for rosette-forming ability. Patients with tumours have a significantly smaller number of E rosette forming lymphocytes ($P<0.01$) compared to patients in remission. This finding is in agreement with the observed impairment of outaneous reactivity, and is suggestive of T lymphocyte deficiency. The percentage of EAC rosettes (B lymphocytes) did not differ with respect to clinical status.

The tumours of 18 patients have been studied in vitro. In 9 of these patients, autologous serum caused a significant increase in H^3Tdr uptake by the tumour cells, and it is of interest that 4 of these patients have since died. One of 7 patients with no enhancement by serum of tumour cell growth, has also died. This difference has not achieved significance, and clearly a longer period of follow-up is is required. One of the patients with serum enhancement manifested a lymphocyte cytotoxic effect on her own tumour cells when lymphocytes were incubated with tumour in the absence of serum. When lymphocytes, serum and tumour were

incubated together, however, the cytotoxic effect was lost. The same patient's lymphocytes increased their uptake of thymidine in the presence of mitomycin C treated tumour cells. There is therefore definite evidence of a cell mediated reaction to the tumour, but it was abrogated by the presence of autologous serum.

The group of patients with enhancing serum also had poor reactivity to recall antigens. Out of 28 tests done on 7 patients, there were only 4 positive reactions (14%). Of 24 skin tests performed on 6 patients in whom serum did not enhance tumour growth, there were 8 positive reactions (33%).

In 2 of the 18 patients, lymphocyte transformation occurred using mitomycin C treated tumour cells as antigen. In one of these patients transformation only occurred in the presence of serum (which by itself, did not effect H^3Tdr uptake of mitomycin C treated tumour cell, but did enhance growth of live cells).

Lymphocyte uptake of H^3Tdr was impaired in the presence of tumour or serum in 5 patients. Three of these patients have died and two have relapsed, but have short follow-up periods at present. Three of the patients had no reactivity to cutaneous recall antigens at the time of in vitro testing, but PHA reactivity of the group as a whole was normal, indicating that the initial phase of lymphocyte function was not impaired (median stimulated : unstimulated = 160). One patient did however have very poor reactivity (ratio S:U = 3.2).

We can conclude from these studies that the host/tumour relationship is very complex. As well as antitumour antibodies, serum could contain soluble antigens and antigen/antibody complexes, and these materials may have general, non-specific actions on both lymphocytes and tumour, as well as specific actions. It is pertinent to remember that the plasma membrane of the tumour cells may well have affinities to the normal lymphocyte plasma-membrane so tumour growth may be affected by similar mechanisms that effect lymphocyte proliferation e.g. antibodies to surface antigens and antigen/antibody complexes. This might explain why Burkitt's tumour serum does not only block cell mediated immunity but causes actual enhancement of tumor growth.

Studies of the tumour cell surface. Thirty tumours have been studied for the ability of PHA to enhance uptake of H^3Tdr. In 9 (33%) of the tumours uptake was increased. One tumour was tested twice, having been stored in the interim for about 3 months at - 70° C. in liquid nitrogen. Upon thawing, it still reacted to PHA. This finding indicates that some

Burkitt tumour cells have PHA receptors akin to those of T-lymphocytes. Contamination of the tumour cells with lymphocytes has been minimal, so that the possibility that the response to PHA is related to the presence of lymphocytes is highly unlikely.

At the present time the surface membrane is being further investigated by assaying the propensity of tumour cells to form rosettes with sheep erythrocytes (EAC and E). So far only 3 tumours have been tested, but all three formed both E and EAC rosette EAC rosettes were 11.5%, 31% and 33%, whilst the E rosette formation was noticeably less - 1.3%, 4.7% and 17.6%. None the less, there are now two pieces of evidence that Burkitt tumour cells may have some surface properties related to T lymphocytes, which is interesting since they clearly also have some of the properties of B lymphocytes, i.e. immunoglobulin formation and EAC rosette formation. It is possible that Burkitt's tumour arises from an uncommitted precursor cell which can give rise to either B or T type lymphocytes.

The finding in this laboratory that Raji and P_3J cells produce a material which can cause inhibition of Guinea-pig macrophage immigration, (present in 8 of 10 unconcentrated supernatants, and 8 of 12 thrice concentrated supernatants with similarly concentrated control) is also of relevance to this topic, since it is T lymphocytes which are thought to be responsible for the production of macrophage inhibitory factor.

Clonal origin of Burkitt's Tumour. One intriguing problem in Burkitt's lymphoma concerns the question of whether the tumour is multicentric, or whether separate tumours have arisen by spread from a single primary. The occasional clinical observation of a treatment response of one tumour in the presence of another unresponsive tumour would suggest that sometimes at least, the cell clones of separate tumours may be different. Dr. Philip Fialkow of the University of Washington has attempted to study this problem by assaying iso-enzyme phenotypes of glucose-6-phosphate dehydrogenase (G-6-PD) and phosphoglyceromutase (PGM). In the case of G-6-PD, (the gene of which is carried on the X-chromosome) according to the Lyon hypothesis of inactivity of one of the X-chromosome in females, heterozygotes should have some cells containing the A iso-enzyme, and some the B iso-enzyme. Thus cells derived from a single clone should be either A or B. Certainly in the heterozygotes female patients studied so far (7) the iso-enzyme in their tumours was essentially of one type. Thus each individual tumour at least appears to be composed of a single cell clone. In one patient two tumours present at the same time were studied, and in another, an initial and relapse tumour were studied. In each case

the tumours were of identical G6PD and PGM phenotypes, suggesting that they originated from the same clone. Clearly more data is required, and the study is likely to continue for some time.

Dr. Fialkow is also studying genetic markers in the blood of our patients in an attempt to determine whether there is a genetic predisposition to the disease, which could of course be related to predisposition to a separate environmental factor, such as malaria.

Evidence of the Presence of Rauscher virus in Burkitt's tumour. For some months now a collaborative study with Drs. Kufe and Spiegelman of Columbia University, New York has been in progress. Not only has Rauscher virus RNA been found in 13 of 15 (87%) of Burkitt tumours (detected by nucleic acid hybridization studies), but this RNA has been found to be associated with reverse transcriptase in a particle consistent with the dimensions of Rauscher virus (determind by ultracentrifugation on a caesium chloride gradient), in 9 of 11 (82%) Burkitt tumours. The next step is to determine whether the tumour cell nuclei contain a DNA which will hybridize with Rauscher virus RNA, suggesting that such DNA was directed by the Rauscher virus RNA, with the help of reverse transcriptase, and perhaps a viral-derived DNA polymerase.

These findings are of great etiologic importance in view of Drs. Kufe and Spiegelmann's recent observation of co-operation between a herpes type virus, and an RNA virus, in the pathogenesis of Marek's disease in chickens. It is possible that a similar situation exists in Burkitt's lymphoma, with Epstein - Barr virus as the helper.

Pharyngeal excretion of Epstein-Barr virus in Burkitt's Lymphoma. Recently a study to determine whether EB virus can be obtained from patients with Burkitt's lymphoma has been set up with Dr. J. Niedermann of Yale University. He has shown the continued excretion of EB virus from patients with invectious mononucleosis for some months after the acute illness. It will be of interest to study EB virus excretion in Burkitt's lymphoma, and determine whether this has any correlation with the clinical course. The assay is dependent upon the induction of blastic transformation in lymphocytes by EB virus, and its prevention to specific antibodies. So far, no results are available.

EB virus related antigens. Studies have continued with one our most long-standing collaborators - Drs. W. and G. Henle of the Childrens Hospital, Philadelphia. Under study at present is the relationship of antibody titres to viral capsid antigen (VCA) and early antigen (EA) to the clinical course. Higher titres of the latter antigen appear to be associated a high risk of relapse but further analysis is required. Also of interest

is the significance of the two types of EA - namely "R" and "D". At present little is known of this.

Dr. Gunven of Dr. G. Klein's Laboratory in the Karolinska Institute, Stockholm has recently commenced the measurement of antibodies to the membrane antigen (MA) which is a separate EBV antigenic system to the others measured by Dr. Henle. The anti-MA titres have been shown to increase with BCG treatment in studies carried out in Nairobi, and Dr. Gunven will determine whether a similar variation occurs in our patients in the randomized BCG trial. At the same time horizontal studies on patients with different types of clinical course are under study in an attempt to determine whether anti-MA titres have clinical significance.

Professor Klein and his associates at the Karolinska Institute, Stockholm have recently developed technique of quantitating EBV genomes in BL cells by means of nucleic acid hybridization. We hope to commence in the near future a study of the relationship of EB virus load to EB virus serology, and also the clinical course.

It is also hoped that Professor Klein will be able to perform antibody coating studies on some of our tumours stored frozen in liquid nitrogen. This may also correlate with clinical course, and with the enhancing effect of autologous serum on tumour cells in vitro noted in our own laboratory in 50% of patients studied.

Other Lymphomas. Studies in other lymphomas have been limited to chemotherapeutic trials, although these patients have often served as controls in the immunological studies of Burkitt's Lymphoma.

In Hodgkins disease, a randomized trial of CCNU maintenance chemotherapy following remission induction by MOPP has been underway for over a year. Results are not yet evaluable. In lymphoblastic lymphoma initial therapy has been with cyclophosphamide, cytosine arabinoside, vincristine and prednisolone (COAP). When patients become resistant to this regimen, methotrexate, vincristine, asparaginase and prednisolone is used, (MOAP) and finally daunomycin is substituted for methotrexate (DOAP) in late refractory patients.

The results with these various regimens are shown below:

	COAP	MOAP	DOAP
No. Patients	7	4	1
Complete Response	3	1	0
Partial Response	4	2	1
No Response	0	1	0

In histiocytic lymphoma, the standard first line treatment has been MOPP. Relapses are treated with CCNU and results are shown below:

	MOPP	CCNU
No patients	6	2
Complete response	3	0
Partial response	3	0
No response	0	2

Childhood sarcomata are treated with alternating courses of COAP and vincristine plus actinomycin D. Of 9 patients treated there have been 2 complete responses, 5 partial responses, and 2 treatment failures.

Tropical Splenomegaly Syndrome. Studies in this disease are now completed, and the major investigative work is being continued under Dr. Jagwe in Mulago Hospital. Carefully controlled serologic studies support an hypothesis attributing the hepatosplenomegaly to reticuloendothedial responses to large circulating immune complexes. Full details of this study will be published shortly.

II. The Solid Tumor Center

Since its opening in December 1969, 487 patients were admitted to the Solid Tumor Center. A comparison of last year's admissions with previous years follows:

DISEASE	1969-71	1972	CUMULATIVE TOTALS
Hepatocellular Carcinoma	98	81	179
Kaposi's Sarcoma	105	57	162
Malignant Melanoma	54	13	67
Hydatidiform Mole	21	6	27
Choriocarcinoma	8	6	14
Miscellaneous	32	6	38
	318	169	487

Explanations for the differences in numbers of patients in each disease category between the first two years and last year reflects some changes in research emphasis at the Solid Tumor Center. Early in 1972 all patients with hepatocellular carcinoma were admitted instead of just "good risk" patients, increasing the number of admissions with that disease; however, as of September, 1972 due to staff shortages of

physicians and research technicians the hepatocellular carcinoma program was discontinued. Additionally, since May 1972 all patients with trophoblastic disease have been transferred directly to the care of the Dept. of Obstetrics and Gynecology, Makerere University Medical School. Thus, at present, only patients with Kaposi's sarcoma and malignant melanoma are admitted to the Solid Tumor Center for protocol studies.

Hepatocellular Carcinoma. In a small series of patients documenting an increased frequency of persistent hepatitis B antigen (HBAg) in the blood of patients with hepatocellular carcinoma has now been confirmed in a larger series of patients. At the present time reanalysis of our serum collection is being done using radioimmunoassay for HBAg and preliminary results indicate that at least 55% of hepatocellular carcinoma patients are HBAg positive. The frequency of antibody to HBAg (anti-HBAg) has also been investigated but no significant differences between hepatocellular carcinoma patients and controls were found. An increased rate of persistent HBAg with no significant differences in anti-HBAg frequency was also described in Ugandan patients with macon dular cirrhosis. It is of further interest that a significant correlation has been found between the presence of persistent HBAg and the presence in Cirrhotic pafients, either with or without coexistent hepatocellular carcinoma, of liver cell dysplasia, a histopathological finding thought to be premalignant. Although these studies provide support for the hypothesis that persistent hepatitis B virus infection plays an etiological role in the ultimate development of hepatocellular carcinoma, other possibilities have been suggested. Immunological studies were done on our patients to test the hypothesis that the high rate of HBAg persistence in these patients is the result of defects in host immune responses. In these studies responses to DNCB sensitization, a battery of recall skin test antigens and the humoral antibody response to tularemia antigen were normal; neither hypogammaglobulinemia nor specific immunologlobulin deficiencies were encourntered. A genetic-environmental study of families of patients with hepatocellular carcinoma is currently being done by Dr. Stephen Lwanga, to test Blumberg's hypothesis "that the distribution of persistent BGAg in families or in populations is compatible with an autosomal recessive mode of inheritance". Also in progress is an analysis of our serum collection for HBAg subtypes.

There are now four potential biochemical markers of disease activity in hepatocellular carcinoma. These include alpha feto-protein (AFP), proline hydroxylase, chorionic gonadotrophin (HCG) and abnormal fibrinogens.

Methodology for AFP measurement continues to be refined. In earlier studies using the standard qualitative double diffusion in agar test and

the more sensitive rivanol precipitation technique we found that sixty-six percent of Ugandan hepatocellular carcinoma patients were AFP positive. It was shown further that the percentage of positive tests are significantly correlated with HBAg and inversely related to increasing age. More recently, research efforts have centered on AFP quantitation using electroimmunodiffusion[11] which is capable of measuring amounts of AFP as low as 350 nanograms/ml and a radioimmunoassay proceedure with limits of detection to less than 25 nanograms/ml.

Serial quantitative AFP determinations in untreated patients with hepatocellular carcinoma have been found to be remarkably constant with time. In contrast, marked decreases in levels were noted in patients undergoing partial hepatic resection. We are currently examining AFP levels in patients who received antineoplastic chemotherapy and are correlating AFP levels with anti-tumor responses and other quantitative markers of disease activity.

Serum protocollagen proline hydroxylose (PPH) levels are elevated in seventy-one percent of patients with hepatocellular carcinoma. While the reasons for this elevation remain obscure this assay appears promising as another potential biochemical marker of disease activity. This possibility is reinforced by the finding of a dramatic fall in PPH levels in one patient experiencing an objective anti-tumor response after intrahepatic arterial chemotherapy. Studies of serial PPH levels in treated and un-treated patients and correlation with other biochemical markers are in progress on stored sera.

Serum HCG levels were found to be elevated in 14% of 125 Ugandan patients with hepatocellular carcinoma. There was a tendency, though not statistically significant, for older AFP negative patients to produce HCG more commonly than younger, AFP positive patients. By following serial quantitative AFP, PPH and HCG levels in treated patients, subclinical evidence of anti-tumor response may be obtained which will assist in the search for effective chemotherapeutic agents for hepatocellular carcinoma.

The fourth potential biochemical marker, abnormal fibrinogen, has not been evaluated as fully as the other three. At present, there is only the suggestion from a detailed study of coagulation and fibrinolysis done in patients with hepatocellular carcinoma that dysfibrinogenemia may be more common in this disease than previously suspected.

A detailed study of the ultrastructure of hepatocellular carcinoma has been completed and published. Most significant was the absence of virus or virus-like particles in any of the specimens studied. In light of the finding of persistent HBAg in the serum of 55% hepatocellular carcinoma patients, continued ultrastructure studies with special emphasis on

in vitro cultures and identification of virus particles therein, appear warranted.

At the International Symposium on Liver Cancer held in Kampala in July 1971 it was felt that one of the prerequisites for future controlled chemotherapeutic trials in hepatocellular carcinoma was the establishment of a universally accepted staging system. Such a system was defined at the Symposium and the prognostic validity of its component parts tested in untreated patients at the Solid Tumor Center. On the basis of this prospective evaluation of the proposed staging system a simpler and more prognostically accurate system was devised.

Thirty-six of the first 120 patients admitted underwent a surgical procedure, usually in the hope of finding a resectable lesion. Partial hepatic resections were done in 10 patients, and hepatic artery catheterization in 11; in the remainder none of these therapeutic attempts was feasible. The results of surgery in these patients have been published as have preliminary results of hepatic artery catheterization in the first three patients treated with dichloromethotrexate. More recently, just prior to discontinuation of the hepatocellular carcinoma program, a pilot study of hepatic ligation was begun by Professor Kyalwazi and Dr. William Rambo. This procedure was done in a total of 7 patients of whom four died, two immediately post-operatively and two within the first two months after surgery. The overall impression of these preliminary studies indicate that at present, surgery can afford direct benefit to only a very small percentage of Ugandan patients with hepatocellular carcinoma.

Initial chemotherapeutic trials with systemically administered dichloromethotrexate were discouraging. In subsequent trials of CCNU and a BCNU-5 FU combination no objective anti-tumor response was noted. It is possible that when final analysis of the data is done and correlated with effects on the three quantitative markers of disease activity, some subclinical evidence of tumor destruction may be detected.

Kaposi's Sarcoma. A clinico-pathological classification has been devised delineating four major clinical and three histological types of disease. Two patients with Kaposi's sarcoma complicating pregnancy were the subject of separate case reports. In earlier chemotherapeutic studies only patients with easily evaluable "florid" lesions were treated but current protocols include all types of tumors with stratification in each randomization scheme by clinical and histopathological type. The first randomized clinical trial established the superiority of actinomycin-D (ACTD) over cyclophosphamide in florid tumors. A subsequent trial suggestively showed that a combination of ACT-D plus vincristine (VCR) was superior to ACT-D alone in florid tumors. This study also demonstrated

the importance of taking tumor histopathology into account when randomizing patients to different treatment protocols, since mixed cellular tumors were found to respond better to therapy than eit her monocellular or anaplastic tumors. Patients refractory to either ACT-D or the ACT D-VCR combination have received a variety of agents in phase II trials. The most effective of these has been dimethyl imidazole carboxamide triazeno (DTIC, NSC 45388) which produced a 50% objective response rate and BCNU (45% response rate). Bleomycin, although producing an objective response rate of 60% produced tumor regressions which were transient.

Currently, new patients are randomized to receive ACT D plus VCR with or without concommitant DTIC. Results are, at present too preliminary to report.

In vivo immunological studies in patients with Kaposi's sarcoma have been reported previously as have studies of in vitro lymphocyte transformation. Preliminary tissue culture studies done in collaboration with Dr. G. Giraldo (Institut de Recherches sur les maladies du sang, Paris, France) have been completed and several cell lines have been established in long term culture. Herpes-type virus were seen in electron micrographs of cultured cells from three of our patients. Comparative immunologic studies showed a common, precipitating, soluble antigen against human reference sera with high anti-cytomegalovirus, low Ebstein-Barr virus titers.

Malignant Melanoma. A summary of the Ugandan experience with malignant melanoma was summarized in 1968 for the period prior to the opening of the Solid Tumor Center and the Solid Tumor Center experience in the initial 56 patients has been recently published. Since April 1972, however, there have been no malignant melanoma admissions. The reasons for this remain unexplained.

Patients with disseminated or inoperable malignant melanoma have been treated in successive Phase II clinical trials first with DTIC (8% response rate) and most recently with imidazole mustard. There have been no objective anti-tumor responses among eight patients with the latter agent. Two patients relapsing after BCG immunotherapy have been treated recently with a three drug combination including DTIC, BCNU and vincristine but it is too soon to evaluate response.

Preliminary in vivo immunologic studies indicated that Ugandan melanoma patients had immune responses similar to control patients. Then, in a preliminary report, a correlation between positive skin tests to antigens prepared from autologous tumor, and tumor stage was suggested. An expanded study in a larger number of patients, however, failed to confirm this preliminary observation.

Preliminary immunotherapy studies established that Pasteur Institute BCG administered by scarification stimulated cell-mediated immune responses. Additionally, it appeared that such treatment might have beneficial effects as an adjunct to surgery in patients whose disease has not spread beyond the regional lymph nodes. More recent followup of the patients in this preliminary study reveals that only one of six patients treated with BCG scarification developed recurrent tumor, one year after completion of immunotherapy. One other patient died of causes not thought to be tumor-related. All other comparable patients treated with immunotherapy using a Glaxo BCG preparation administered intradermally relapsed within 6 months of treatment. As a result of this preliminary study all patients with operable tumors confined to an extremity, with or without regional lymph node involvement (Stage I and II) were randomized after local excision and regional lymphadenectomy to receive: (1) BCG scarification (2) adjunctive CCNU chemotherapy or (3) no therapy. Following is a summary of patients entered on study. Survival information of the four patients still in remission from the preliminary study are included.

Experience with Scarification BCG Immunotherapy

First Study

Pt. Name	Lymph Nodes	Regimen	Type and Months to Relapse	Months Survival from Diagnosis
Y. Aku	Pos	BCG	-	24 +
L. Nam	Neg	"	-	32 +
Kap	Pos	"	-	30 +
Kag	Pos	"	-	29 +

Second Study

Kar	Pos	BCG	9 (Local)	13 +
Mug	Pos	"	12 (Local)	13 +
And	Pos	"	-	10 +
Kat	Neg	"	12 (Local) Distant)	25 +
Mus	Neg	"	-	22 +
Mas	Neg	"	-	8 + (lost)
Nan	Pos	CCNU	-	15 +
Mut	Pos	"	2	3
Kal	Pos	"	13	12 +
Kagu	Neg	"	-	*6 + (lost)
E.A.Nam	Neg	"	11 (Local)	16 +
Mal	Neg	"	-	*9 + (lost)
Nak	Pos	Placebo	-	21 +
But	Pos	"	12 (Local)	16 +

Almost 20% of our patients with malignant melanoma have developed vitiligo during the evolution of their malignant melanoma. Immunologic mechanisms of an auto-immune type have been hypothesized to explain this phenomenon; alternatively local and systemic changes in melanoma metabolism might be involved. In collaboration with Dr. S. Klaus, Yale University Medical School, electron microscopic and enzyme studies have been done to help elucidate the reasons for this association however final results are not yet available. Further studies of pigment metabolism in melanoma tissue have been done in collaboration with Professor Rorsman of the University of Gothenburg, Sweden. Preliminary studies have shown that four out of seven melanoma tissue specimens studied contained 5-S-Cysteinyldopa. The presence of this substance may be an indication of an aberrent metabolic pathway in melanoma tumor tissue. Alternatively, 5-S-Cysteinyldopa may be produced during normal melanoma formation in Africans as well as in red-headed Caucasians. Further studies aimed at resolving this point should be undertaken.

Honors and Awards:

John L. Ziegler, M.D.

1. Albert and Mary Lasker Award for Clinical Cancer Chemotherapy, November, 1972.

2. "Burkitt's Lymphoma" presented at the University of Wisconsin Medical School and Veterans Administration Hospital, Madison, Wisconsin, February, 1973.

3. "Chemotherapy of Burkitt's Lymphoma" presented at the National Conference on Chemotherapy, New York, June, 1972.

4. "BCG Immunotherapy in Burkitt's Lymphoma" presented at the Conference on the Use of BCG in the Therapy of Cancer, NIH, Bethesda, Maryland, October, 1972.

Charles L. Vogel

1. "Hepatocellular Carcinoma in Uganda" presented at the International Conference on Liver Cancer, Capetown, South Africa, January, 1973.

2. U.S.P.H.S. Commendation Medal, January 1973.

Publications:

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5. Magrath, I. T., Lwanga, S., Carswell, W., Harrison, N.: Factors effecting prognosis in abdominal Burkitt's lymphoma and the role of surgical reduction of tumour bulk. Brit. J. Surg. (In press).

6. Magrath, I. T., Ziegler, J. L., and Templeton, A.S.: A comparison of clinical and histopathological features of childhood malignant lymphoma in Uganda. Cancer. (In press).

7. Magrath, I. T., Ziegler, J. L., Bluming, A.Z.: Preliminary results of a randomized trial of BCG immunotherapy in Burkitt's lymphoma. Proc. of Paris Cancer Week. (In press).

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9. Ziegler, J. L., Bluming, A. Z., Fass, L., Magrath, I. T., and Templeton, A. C.: Chemotherapy of childhood Hodgkin's disease. Lancet 2: 679, 1972.

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13. Ziegler, J. L., Voller, A., and Ponnudurai, T.: Malarial antibodies in tropical splenomegaly syndrome in East Africa. Trop. Geogr. Med. (In press).

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15. Ziegler, J. L., Magrath, I. T. and Bluming, A. Z.: Approaches to the management of Burkitt's lymphoma involving the central nervous system. Afr. J. Med. Sci. (In press).

16. Ziegler, J. L. and Stuiver, P.: Tropical splenomegaly syndrome in a Rwandan kindred in Uganda. Brit. Med. J. 3:37, 1972.

17. Ziegler, J. L., Bluming, A. Z., Fass, L. and Morrow, R. H.: Relapse patterns in Burkitt's lymphoma. Cancer Res. 32:1267, 1972.

Hepatocellular Carcinoma.

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Vogel, C. L., Anthony, P. P., Sadikali, F. Barker, L. F. and Peterson, M. R.: Hepatitis-associated antigen and antibody in hepatocellular carcinoma: Results of a continuing study. J. Nat. Cancer Inst. 48:1583, 1972.

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Anthongy, P. P., Vogel, C. L. and Barker, L. F.: Liver cell dysplasia: A pre-malignant change. J. Clin. Path. (In press).

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Vogel, C. L., Adamson, R. H., DeVita, V. T., Johns, D. C. and Kyalwazi, S.K.: Preliminary clinical trials of dichloromethetrexate in hepatocellular carcinoma. Cancer Chemother. Rep. 56:249, 1972.

O'Connor, G. T., Tralka, T. S., Henson, E. and Vogel, C. L.: Ultra-structural survey of primary liver cell carcinoma from Uganda. J. Nat. Cancer Inst. 48:583, 1972.

Primack, A.: The management of hepatocellular carcinoma at the Uganda Cancer Institute. Uganda Med. J. 1:41, 1972.

Harrison, N. W., Dhru, D., Primack, A., Bhana, D. and Kyalwazi, S. K.: The surgical management of primary hepatocellular carcinoma in Uganda. (submitted).

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Kaposi's Sarcoma:

Taylor, F. J., Templeton, A. C., Vogel, C. L., Ziegler, J. L. and Kyalwazi, S. K.: Kaposi's sarcoma in Uganda: A clinicopathological study. Int. J. Cancer 8:122, 1972.

Vogel, C. L., Primack, A., Dhru, D., Briers, P., Owor, R. and Kyalwazi, S. K.: Treatment of Kaposi's sarcoma with a combination of actinomycin-D and vincristine: Results of a randomized clinical trial. Cancer. (In press).

Vogel, C. L., Primack, A., Owor, R. and Kyalwazi, S. K.: Effective treatment of Kaposi's sarcoma with 5- (3,3,-dimethyl 1-triazeno) imidazole - 4 - carboxamide (DTIC, NSC, 45,388) Cancer Chemotherapy Rep. (In press).

Vogel, C. L., Clements, D., Wanume, A. K., Toya, T., Primack, A. and Kyalwazi, S. K.: Phase II Clinical trials of 1,3-Bis (2-chloroethyl - 1 - nitrosoure. (BCNU, NSC 4099621) and Bleomycin (NSC, 125066) in Kaposi's sarcoma. (submitted).

Giraldo, G., Beth, E., Coeur, P., Vogel, C. L., and Dhru, D. S.: Kaposi's sarcoma: Tissue culture, virologic and immunologic studies. J. Nat. Cancer Inst. (In press).

Malignant Melanoma:

Gallico, G. G., Vogel, C. L., Kiryabwire, J. W. M., Primack, A., Dhru, D. and Kamya, G. W. S.: Recent advances in malignant melanoma research in Uganda. Uganda Med. J. (In press).

Primack, A., Dhru, D., Kiryabwire, J. W. M., and Vogel, C. L.: Clinical trials of combination chemotherapy with BCNU and vincristine in the treatment of malignant melanoma in Uganda. Cancer (In press).

Bluming, A. Z., Vogel, C. L., Ziegler, J. L. and Kiryabwire, J. W. M.: Delayed cutaneous reactions to extracts of autologous malignant melanoma: A second look. J. Nat. Cancer Inst. 48:17, 1972.

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Serial No. NCI-6810
1. Pediatric Oncology
2. Medical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 - June 30, 1973

Project Title: Chemo-immunotherapy

Previous Serial Number: Incorporating previous numbers NCI 6302, NCI 3400

Principal Investigator: Brigid G. Leventhal, M.D.

Other Investigators: John H. Bryan, M.D., Charles Olweny, M.D., Edward S. Henderson, M.D., Roger H. Halterman, M.D., John L. Ziegler, M.D., W. A. Bleyer, M.D., J.Glick, M.D., D. L. Mann, M.D., R. B. Herberman, M.D., D. Char, M.D., R. Simon, Ph.D., R.G. Graw, M.D., A. S. Levine, M.D., G. Johnson, M. J. Sutherland, M.A. Rankin, S. Treim, R.N., N. Keyes, R. N.

Cooperating Units: Immunology Branch, NCI
Cellular & Tumor Immunology Section, LCB, NCI

Man Years:

Total: 14 ½
Professional: 9 (excluding 1st year Clinical Associate)
Other: 5 ½

Project Description: Linear Array Code - IV 4X1, V 5XX, VI 6X1, VII 7XX

Objectives:

1) To detect, characterize and purify specific tumor antigens in acute leukemia.

2) To assess the effect of chemotherapy and immunotherapy on the immune response in general.

3) To assess the effect of chemotherapy and immunotherapy on the specific response to tumor antigen.

4) To analyze the elements of the immune response associated with prognosis in acute leukemia.

5) To devise therapeutic protocols which will allow maximal tumor cell kill with drugs while leaving intact effective elements of the anti-tumor immune response.

Progress:

A. Detection and characterization of acute leukemia antigen.

It was noted last year that after immunization with RAJI membrane fragments, rabbits develop an antiserum which is cytotoxic to acute leukemia cells. These studies were extended to show that lymphoblasts from patients in relapse were lysed by the antiserum while marrow cells from the same individuals in remission were unaffected, and thus reactivity to HL-A antigens was not responsible for the reactivity. In vitro studies by Dr. Mann also demonstrated that this antiserum lyses only human lymphoid cells in tissue culture, although susceptibility to lysis could be induced in human embryo kidney cells after infection with Rauscher virus. These studies suggest that the antigen detected by the rabbit anti-RAJI antisera is a human antigen expressed on lymphoid and myeloid cells after de-differentiation.

B. Effect of chemotherapy and immunotherapy on the immune response in general. Studies are in progress assessing immunoglobulin levels, short term lymphocyte culture response, E rosette formation, and skin test reactivity in patients receiving chemotherapy. In general we have concluded that patients receiving methotrexate alone or POMP combination show a rebound in T-cell response 10-15 days after therapy while the COAP combination appears to produce more consistent and prolonged immunosuppression. In the course of these studies it has become obvious that different T-cell assays are affected to varying degrees by chemotherapy. PHA response falls and then reaches a peak after therapy at the same time that rosette formation reaches a nadir, while direct lymphocyte cytotoxicity is not affected consistently by chemotherapy. These findings have implications for basic immunology in that they point to heterogeneity in the T-cell population with differential drug sensitivity of the T-cell subpopulations.

C. Analysis of the elements of the immune response associated with a good prognosis.

In several studies it has become clear that positive delayed hypersensitivity responses to tumor antigen are associated with prolonged remission duration. In one study (NCI 72-2) it was also found that vigorous MLC responses to autologous tumor was associated with prolonged remission duration. Thus, a positive correlation between immune

recognition of tumor antigen(s) and remission duration was demonstrated.

D. Chemo-immunotherapy protocols.

1. NCI 72-1. Previously untreated children under 20 years of age with ALL are induced into remission with POMP. They then undergo CNS prophylaxis with 2400 r to the skull and intrathecal chemotherapy with either methotrexate or cytosine arabinoside plus systemic hydroxyurea (first randomization). The aim of this randomization is to evaluate the combination of cytosine arabinoside and hydroxyurea in search of regimen as effective and yet less toxic than methotrexate. After consolidation therapy is complete patients are placed on maintenance which consists of two phases given alternately. Phase I is a four month course of chemotherapy with daily 6-MP, weekly methotrexate and three weekly pulses of vincristine and prednisone at months 1 and 3. At Phase II the patients receive either more methotrexate given twice weekly or in 5-day pulses, or BCG plus allogeneic cells (second randomization). Immune evaluation is performed on all patients during Phase II. Phase I and II are then alternated 3½ more times. To date 53 patients have been entered on this protocol which was begun in January, 1971. Two are too early to evaluate and 48/51 (94%) achieved complete remission. Ten of these patients have terminated remission with bone marrow relapse and two additional patients have developed meningeal leukemia while still in marrow remission. The median remission duration for the group as a whole is 315+ days. Thirty three patients have completed consolidation and have been randomized to one of the two methotrexate schedules or to BCG + cells. To date relapse has occured in 2/10 receiving 5-day methotrexate 1/11 receiving bi-weekly methotrexate, and 2/12 receiving BCG plus cells. No differences have appeared in either of the randomizations on this protocol to date. Since it appears that the two methotrexate schedules are probably equivalent, we plan to drop the 5-day courses of methotrexate and randomize patients for maintenance either to bi-weekly methotrexate or immunotherapy.

2. Twenty-five patients with previously treated acute lymphatic leukemia were given asparaginase either alone or in combination with actinomycin D to induce remission. Sixteen achieved remission and were randomized to maintenance therapy with either weekly BCG plus allogeneic leukemia cells (9 patients) of methotrexate in five day pulses plus allogeneic cells (7 patients). All patients subsequently relapsed, and the remission duration was equivalent in both groups, 91 and 151 days respectively. There was evidence of specific sensitization to tuberculin both in vivo and in vitro in the BCG treated group. There was evidence of an increase in reactivity to recall antigens (candidin, SKSD, and mumps) in both groups on skin testing.

In vitro, the methotrexate treated group showed a cyclic increase in lymphacyte response to antigens after each course of methotrexate. The response to immunizing cells followed the same pattern in vivo and in vitro as the response to recall antigens. Those individuals showing positive skin reactivity to immunizing cell membrane preparations or vigorous in vitro blastogenic response to autologous leukemia cells had longer remissions than those who were non-reactive in these assays. These data suggest that reactivity to leukemia antigen is associated with a prolonged remission even in a patient with relatively advanced disease, but that immune manipulation produced no observable therapeutic differences between the two groups.

3. NCI 73-4. This protocol was designed to further explore the earlier observation that there appears to be a common antigen on the surface of RAJI and acute leukemia blast cells. Patients with previously treated ALL and AML in relapse are put into remission with asparaginase plus vincristine, daunomycin and prednisone for ALL or 3-day courses of daunomycin for AML. After induction, the patients are randomized to maintenance with COAP alone or COAP + RAJI cell injections. To date 16 patients are evaluable on this protocol. It is too early to observe an effect on remission duration, but the immunization schedule does appear to be effective in inducing cytotoxic antibody to RAJI cells.

E. Chemotherapy protocols.

1. 72-5 Meningeal Leukemia. Patients with meningeal leukemia regardless of age or basic diagnosis were randomized on this study to receive intrathecal methotrexate with or without CNS radiation. The first 17 patients were also randomized to receive methotrexate with or without preservative. Thirty-one patients with meningeal leukemia were randomized to therapy with intrathecal methotrexate with and without 2400 r to the cranial vault. Remissions were achieved in 29 patients. Remission duration was significantly longer (234+ days) in the group receiving MTX alone than in those receiving MTX + X-ray (125 days) ($p < .05$). A spectrum of methotrexate toxicity was seen after intrathecal administration ranging from chemical arachnoiditis which was common (17 patients) to transient paresis (1 patient) or encephalopathy (2 patients). Toxicity was as common in patients receiving preservative-free methotrexate as in those receiving methotrexate with preservative.

2. 72-4 Ara-C 6-TG in advanced ALL. Nine of 19 patients with late refractory ALL achieved brief remissions after therapy with Ara-C - 100 mg/m^2 s.c. and 6-TG 90 mg/m^2 p.o. every 12 hours given until marrow hypoplasia developed. This is an effective regimen in ALL and has

been included in the early phase of therapy.

3. 72-13 Long-term toxicity from chemotherapy in acute leukemia. Twenty-four patients in continuous remission for 36-120 months were evaluated comprehensively. Diminished marrow iron stores were noted in 22/24 but there were no clinically apparent abnormalities of erythropoiesis; marrow function was otherwise normal. Four patients had elevated BSP retention including one who had sustained an episode of hemorrhage from esophageal varices and documented cirrhosis. Three others with normal liver function studies had biopsy proven drug hepatitis in the past. Decreased or absent ankle jerks were seen in 9/24 but no patient was functionally incapacitated with VCR toxicity. Five had mild elevations in CSF pressure and 6/24 had mild posterior subcapsular cataracts. Both of these findings were ascribed to high dose steroid therapy. There were no significant renal, cardiovascular, pulmonary or endocrine abnormalities and growth and development were within the usual range. Thus, despite mild abnormalities within several organ systems, the group as a whole showed minimal long-term toxicity from intensive, prolonged chemotherapy.

4. 72-7 Methyl CCNU. Seventeen patients with ALL have been treated with 2 partial responses. One patient with AML, age 15, achieved complete remission at 250 mg/m^2.

Conclusions:

Tumor associated and/or tumor specific antigens are detectable on human leukemia cells. Antigens common to acute leukemia and RAJI cells provoke the formation of cytotoxic antibody in rabbits and in man and the therapeutic possibilities of this discovery are being explored. Immune reactivity to tumor antigens is associated with a good prognosis even in late stage ALL. To date, however, controlled manipulation of this immune response has not been therapeutically effective.

Proposed course of the project:

Major emphasis during the coming year will be on completion of two acute leukemia protocols. One (73-4) to evaluate the effect of RAJI immunization on remission duration and the other to analyze the adjuvant effect of BCG (72-1). The adult acute leukemia program is to be phased out and further patient studies are planned in other pediatric tumors which lend themselves to a combination of chemo and immunotherapy.

Honors and Awards:

Brigid G. Leventhal

Member, Program Committee, American Society of Clinical Oncology. Member, Master's Thesis Committee, Department of Chemistry, American University, for Mr. Mark Schlam.

Invited Lectures:

Brigid G. Leventhal

1. Semaine de Cancerologie, CNRS, Paris, France, June 1972.
2. Hematology Board Review Course AFIP and George Washington Medical School, 1972.
3. Mixed Leukocyte Culture Workshop, Madison, Wisconsin, September, 1972.
4. Current Concepts in the Treatment of Malignant Disease, NCI, May, 1972.
5. Seminar for Grantees, Immunology Program, NCI. Woods Hole, Mass, October, 1972.
6. Symposium on BCG, NCI, October, 1972.
7. Georgetown University Hematology Seminar, October, 1972.
8. Conference on Immunochemotherapy. Mt. Sinai Hospital, November, 1972
9. Histochemistry in Clinical Hematology. Faculty of Histopathology, AFIP, December, 1972.
10. Immunotherapy of Acute Leukemia. Children's Cancer Research Foundation, Boston, Massachusetts, November, 1972.

Publications:

1. Herberman, R. B., Rosenberg, E. B., Halterman, R. H., McCoy, J. L., and Leventhal, B. G.: Cellular immune reactions to human leukemia. Immunology of Carcinogenesis conference, Gatlinburg, Tennessee. May 1972. NCI Monograph 35: 259-266, 1972.
2. Halterman, R. H., Leventhal, B.G., and Mann, D.C.: A leukemia associated antigen: Its relationship to clinical status. New Eng. J. Med. 287: 1272-1275, 1972

3. Herberman, R.B., Hollinshead, A.C., Alford, T.C., McCoy, J. L., Halterman, R. H. and Leventhal, B.G.: Delayed cutaneous hypersensitivity reactions to extracts of human tumors. JNCI (In Press).

4. Leventhal, B.G., Halterman, R. H., Rosenberg, E. B., McCoy, J. L. and Herberman, R.B.: Lymphocyte cytotoxicity in human acute leukemia. Semaine de Cancerologie, Paris, 1972. C.N.R.S. (In Press).

5. Leventhal, B.G., Le Pourhiet, A., Halterman, R. H., Henderson, E.S. and Herberman, R. B.: Immunotherapy in previously treated acute lymphatic leukemia. Symposium on BCG. 1972. JNCI (In Press).

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Serial No. NCI-6820 (c)
1. Experimental Hematology Section
2. Pediatric Oncology Branch
3. Clinical Oncology, Chemotherapy, NCI

PHS-NIH
Individual Progress Report
July 1, 1972 through June 30, 1973

Project Title: Blood Component Transfusion and Bone Marrow Transplantation

Previous Serial Number: NCI-3415, NCI-6301, NCI-3426

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Cooperating Units: Medicine Branch, NCI; Immunology Branch, NCI; Laboratory of Pathology, NCI; Hematology Service, Clinical Pathology, Clinical Center, NIH; Blood Bank, Clinical Center, NIH; Anethesiology Department, NIH; National Institute of Allergy and Infectious Disease, LCI; Department of Surgery, University of California at Los Angeles; Mary Imogene Bassett Hospital, Cooperstown, New York; Department of Medicine and Surgery, Division of Research Services, NIH; Plasmapheresis Unit, NCI; Dermatology Branch, NCI; Radiation Branch, NCI; Case Western Reserve, University School of Medicine, Cleveland, Ohio; Armed Forces Radio-Biology Institute, Bethesda, Maryland; Tissue Bank and Naval Research Institute, Bethesda, Maryland; Microbiological Associates, Bethesda, Maryland; Hazleton Laboratories, Falls Church, Virginia; Bionetics Incorporated, Rockville, Maryland; National Eye Institute, NIH; ABCOR Corporation, Boston, Massachusetts; A.D. Little, Boston, Mass.; Oakridge National Laboratories, Oakridge, Tennessee; Central Blood Service, Baltimore, Maryland;

American National Red Cross, Washington, D.C.

Man Years:

Total: 10
Professional: 6
Other: 4

Project Description: Linear Array Code VI 6x1

Objectives:

1) The provision of effective blood component transfusion therapy for patients with depressed bone marrow function, in particular emphasizing the development of improved procurement and transfusion techniques for blood platelets and granulocytes.

2) Evaluation of histocompatibility antigen variables as they govern transfusion responsiveness in patients alloimmunized to transplantation antigens.

3) Assessment of the optimal methods for obtaining and maintaining allogeneic, autologous and isogeneic bone marrow grafts.

4) Examination of the utility of bone marrow transplantation and other forms of immunologic manipulation in the treatment of large animals spontaneous neoplasms (canine).

Methods Employed:

1) Transfusions of platelets and leukocytes on the Clinical Oncology Service continued to be the major means of evaluating new transfusion techniques. A large donor panel has been HL-A typed (at least 3,500 individuals) and these individuals are available for donation of platelets or granulocytes. Platelets have been collected using 4-unit plateletpheresis techniques or the Lathum plateletpheresis machine. Granulocytes are collected using the NCI continuous flow Blood Cell Separator or Filtration Leukopheresis. All results of procurement and transfusion studies have been computerized for evaluation.

2) Bone marrow transplantation studies have been performed with terminal leukemic patients, utilizing techniques described in previous years.

3) Pre-clinical leukocyte transfusions, bone marrow transplantation experiments as well as spontaneous tumor models have been established in the dog.

4) A primate (rhesus monkey) model system is now also being employed to evaluate basic transplantation phenomena other than those being studied in the dog.

Major Findings:

1) Major emphasis in the platelet transfusion studies has centered about the question whether HL-A typing is an adequate "match" for effective and reproducible transfusion responsiveness. Using the above described donor pool, HL-A typed blood products were transfused to patients who were alloimmunized to random donor blood products. These studies have indicated that HL-A typing can be effective and reliably used to select donors when three or four HL-A antigens are defined in the recipients HL-A phenotype. In cases where poor antigen definition occurs, it remains impossible to find suitable platelet donors. Using the platelet complement fixation assay, it was possible to identify certain platelet donors who lacked HL-A antigens (of certain specificities) on their platelets. This observation is extremely important since it infers that some donor platelets will circulate despite lymphocyte antigen incompatibility. The use of the serotonin assay (radio-labeled isotope) for platelet antibody has made possible the identification of some HL-A matched donors whose platelets produce better increments. In these studies, recipient serum fails to damage platelets of the compatible HL-A matched donors. The patients demonstrating such antibody have circulated only those platelets to which they have no platelet directed antibody. Leukoagglutination studies in alloimmunized patients have shown the regular development of leukocyte agglutinating and granulocyte-toxicity antibodies early in the course of alloimmunization. Platelet concentrates contaminated with large quantities of leukocytes failed to circulate and are associated with severe transfusion reactions. Preliminary studies indicate that additional processing of platelet concentrates by various specific centrifugation steps can further reduce the leukocytes contaminating collected platelets units and subsequently reduce the incidence of undesirable transfusion reactions. Procurement studies have been continued using the Lathum plateletpheresis machine in order to evaluate whether a larger centrifuge bowl could be employed to reduce the frequency of single donor plateletpheresis.

2) Granulocyte transfusion studies have continued to compare the effectiveness of cells collected using the two above described techniques. Cells collected by Filtration Leukopheresis continue to be associated with severe transfusion reactions, poor intravascular survival, and donor procurement reactions. Animal studies have been expanded indicating that cells collected using nylon fibers, glass beads, or nylon beads fail to circulate normally. However cells collected on glass bead columns circulate somewhat better than those collected on nylon fibers. Human procurement studies have emphasized new leukopak column elution techniques but all modifications have failed to alter transfusion effectiveness. Despite the poor intravascular survival of filtration leukopheresis collected granulocytes, patients receiving the cells have demonstrated recovery from gram negative bacterial infection. Using cells collected with either technique, ninety percent of patients receiving continuous granulocyte transfusion therapy initiated soon after the documentation of gram negative bacterial

sepsis have recovered as reported last year. Donors selected from the random donor file and matched for HL-A antigens have been successfully used in instances where family members were not available. In some instances despite HL-A matching of donor and recipient, a leukoagglutination reaction was observed when donor cells were incubated with recipient serum. This reaction may signal the presence of non-HL-A antigen reactivity in the donor serum. Initial granulocyte antigen typing was performed this year but has failed to demonstrate differences between granulocyte antigen types of donors and recipients having positive leukoagglutination reactions. A microgranulocytotoxicity assay developed at UCLA was employed to examine the incidence of granulocyte antibodies in our patient population. It appears that antibodies directed against granulocyte specificities are more common than those directed against lymphocyte antigens and that they segregate with a different reaction pattern than with HL-A antigens. Attempts to refine the current leukoagglutination techniques (slide and microcapillary) to increase the sensitivity for screening recipient serum for donor compatibility were unsuccessful. Other assays attempted to examine the inhibition of cellular functions (i.e. phagocytosis or bactericidal activity) were also unsuccessful. Modifications to the in vivo processing of blood through the continuous flow Blood Cell Separator was once again studied using "feedback" tubing systems in an effort to verify an observation made with chronic myelogenous leukemic donors that recirculation of packed red blood cell and plasma fractions would improve the collection efficiency. All controlled studies performed to date revealed no change in granulocyte collection. A centrifuge bowl interface controller developed by ABCOR Incorporated now permits the manipulation of the red blood cell-plasma interface on the continuous flow Blood Cell Separator with minimal technician handling. It is anticipated that this instrumentation will permit a more precise collection using the continuous flow Blood Cell Separator. If preliminary studies prove consistent, all instruments will be modified with the new controller. Preclinical leukocyte transfusion studies have emphasized the development of a model for gram negative bacterial pneumonias. Post-irradiation (350 rads) animals were innoculated with pseudomonas (10^7 organisms) into the right main stem bronchial tree when their granulocyte count was < 500 per cu mm. All animals not given granulocyte transfusion support died with pneumonias and bacteremia. Animals treated with granulocytes collected with the Blood Cell Separator have shown marked improvement. In four paired experiments (control and transfusion recipient run simultaneously), animals receiving 7 consecutive daily granulocyte transfusions (begun 24 hours following bacterial inoculation) were able to have significant prolongation of life with two animals completely recovered. These animal studies have carefully documented parameters of infection such as pulmonary macrophage function, antibiotic levels, endotoxin levels, quantitative blood cultures, pseudomonas serotype and host antibody response to the organism, and will provide a basis for many additional studies which will have direct bearing on immunodeficient cancer patients. This animal model will be expanded to incorporate an analysis of leukokinetics in animals transfused with cell separator versus Filtration Leukopheresis

cells. No animals receiving leukocyte transfusions post-irradiation developed leukoagglutinating antibodies to donor granulocytes.

In collaboration with the Armed Forces Radio-Biology Institute it has been shown that animals given sublethal total gamma radiation (350 rads) could survive when given intensive support with antibiotics, parenteral fluids, irradiated whole blood, and platelet concentrates (8/9 survivors versus 0/7 in control group). Animals similarly irradiated were shown to be immunologically responsive being capable of developing an antibody against transfused granulocytes and heterologous erythrocytes which were given post gamma radiation. Whether this model of post irradiation transfusion can be used to evaluate enhanced immunoreactivity to transfused blood products remains unexamined. Studies now in progress are aimed at examining the role of bowel flora on post irradiation mortality.

3) Clinical bone marrow transplantation has been continued using the immunosuppressive regimen developed last year. Three patients with acute lymphocytic leukemia each received six consecutive days of B.A.C.T. which includes Cyclophosphamide (45 mg per kilo per day); Cytosine Arabinoside (100 mg per square meter q 12 hours) x 11 doses; 6-Thioguanine (100 mg per square meter q 12 hours) x 11 doses; and BCNU (200 mg per square meter intravenously) as a single dose on day 3. One patient expired on day 3 with progressive myocardial dysfunction and the post-mortem revealed myocardial damage secondary to underlying daunorubicin therapy and the recent cyclophosphamide. The second patient with acute lymphocytic leukemia lived 32 days following engraftment and died with progressive graft-versus-host disease despite attempts to manage with post-transplant cyclophosphamide. The third patient was successfully engrafted despite major ABO antigen Groups incompatibility, (A→O), and lived 115 days succumbing to infectious complications attributed to previous graft-versus-host disease of moderate severity. This patient also had received cyclophosphamide during the post-transplant period. All patients were HLA genotypically identical to their sibling bone marrow donors and in each case HLA matching was confirmed by a negative mixed leukocyte culture response. One patient with blastic phase of chronic myelogeneous leukemia received four consecutive days of B.A.C.T. in combination with several doses of Hydroxyurea in preparation for an HLA and MLC identical bone marrow transplant. This regimen failed to irradicate residual tumor tissue in this patient identified by the presence of a persistent Philadelphia chromosome in psot-transplant marrow aspirates. This patient is presently being prepared for a second marrow transplant attempt. Two identical twin patients, one with acute myelogeneous leukemia (17 years old) and one with acute lymphocytic leukemia (4 years old) received identical twin marrow transplants following preparation with four-day B.A.C.T. chemotherapy. In each case, there was complete irradication of tumor tissue and return to normal hematopoiesis following engraftment. Both patients remain in clinical remission at four months and six weeks post-transplant respectively. Two patients were transfused with frozen autologous bone marrow following irradiation of tumor tissue using four-

day B.A.C.T. In each case, the patients were in their terminal phases of malignant relapse and had been ill with fevers and infections. Both patients were difficult to support during the post-drug period being refractory to random donors. One patient survived 30 days post-marrow transfer but succumbed to candida albicans meningitis. No post-mortem was permitted. The second patient succumbed at 14 days post-transplant also of overwhelming candidiasis despite being placed in the laminar air flow isolation facility. In summary, the use of the B.A.C.T. chemotherapy regimen has greatly facilitated tumor irradication in terminal leukemic patients. In each case where marrow engraftment was successful, there was complete donor chimerism established. In no instance was mixed chimerism identified. The one toxic death related to the six day protocol was similar to cases reported by other institutions using cyclophosphamide alone. Because of this toxicity subsequent patients during the year received only the four-day protocol. If the four-day regimen shows no myocardial toxicity patients will once again be given the six-day version. No patient received antilymphocyte serum for graft-versus-host disease because of the inavailability of this agent. It is strongly felt that the patient engrafted last year using the B.A.C.T. regimen who was treated with ALS for graft-versus-host disease survived because of the addition of this immunosuppressive agent. This patient, a 19 year old male with acute myelogeneous leukemia, remains in clinical remission for 18 months post-marrow engraftment and has returned to full activities. During the initial post-transplant period however he was plagued by recurrent upper respiratory tract infections and combined immunologic system defects. Over a period of 12 months, these immunologic defects gradually improved as did the patient's clinical course.

In order to evaluate the feasibility of unrelated "matched" marrow donors for marrow transplantation studies, an extensive testing of unrelated HLA matched individuals has been undertaken. To this end approximately 250 individuals with identical 4 antigen HL-A phenotypes are being studied for mixed leukocyte culture compatibility and serologic identity in the HL-A system. Important in these studies will be an evaluation of matching for histocompatibility antigens in the second locus of HLA as well as in the first and second locus combined. This has particular importance since preliminary results in renal transplantation have indicated that matching for the second antigen locus appears to be the most important variable for the success of renal transplants. Presently, 125 individuals have been tested with about 10% incidence of non-stimulation in MLC.

4) Pre-clinical animal studies continue to be extensively used to evaluate various parameters of transfusion and transplantation biology.

   a) The canine marrow allografting model was carefully examined for the variables which contributed to successful and reproducible engraftment without marrow rejection. It is now clearly established that dogs receiving greater than 3.5×10^8/kg of marrow cells following either 1,000 rads (9 rads per minute) or 800 rads (46 rads per minute) will have successful marrow engraftments. If the marrow dose is increased

to 4.5 x 10^8 per kilo (a dosage obtainable in man), this marrow engraftment will be associated with no subsequent rejection. Animals receiving marrow doses between 3.5 and 4.5 x 10^8/kg can be given L-asparaginase (a non-cytotoxic immunosuppressant) to augment the immunosuppression required for permanence of engraftment.

b) "Marrow Rescue" experiments were continued during the early part of the year demonstrating that dogs could survive irradication of unwanted allogeneic bone marrow and reconstitution with frozen autologous bone marrow for the treatment of graft-versus-host disease. This successful management includes intensive hematologic support including platelet and granulocyte transfusions following the institution of cyclophosphamide to irradicate the mismatched bone marrow.

c) Rifampin was administered to several animals post-transplantation of allogeneic bone marrow (prepared with total body gamma radiation) in an effort to evaluate whether rifampin would offer additional immunosuppression similar to that observed with L-asparaginase. Six dogs were transplanted using post-transplant rifampin and only two were successfully engrafted. The post-transplant course was identical to dogs receiving no post-transplant immunosuppression, dying with GvH at 18 and 24 days. It was concluded that rifampin at the dose used in these studies (22 mg/kilogram) offered no additional immunosuppression following marrow transplantation.

d) Mixed leukocyte culture analysis was contined of dog litters raised at the NIH. Many mixed leukocyte culture compatible siblings have been identified and are being followed until they are old enough to tolerate bone marrow transplantation procedures. Additional DLA identical littermates and non-littermates have been purchased from a breeder in New York State. These animals have been DLA typed using antisera raised at the Cooperstown Dog Colony. Mixed leukocyte culture analyses of these "typed" animals reveal several subpopulations. Littermate animals typed to be DLA identical were in the most part confirmed to be identical by negative mixed leukocyte culture response. On the other hand, animals who were thought to be DLA identical non-littermates were for the most part found not to be mixed leukocyte culture compatible. A special group of dogs was purchased from the Cooperstown colony who were known to be genotypically not DLA identical but possessed the identical DLA phenotype. These animals were found to have mixed leukocyte culture stimulation of 50% of the cases. All of these animals will be transplanted to determine the incidence and spectrum of graft-versus-host disease in animals matched for DLA antigens and/or found to have non-stimulation in the mixed leukocyte culture.

e) Initial transplant experiments were performed using DLA matched and MLC non-stimulatory littermates. Seventy percent of these animals have had persistent chimerism demonstrated by chromosome or erythrocyte antigen markers and have not developed the sequelae of graft-

versus-host disease. The remainder of the animals succumbed to protracted GvH and its sequelae. These experiments will eventually serve as controls for attempts to irradicate graft-versus-host disease in animals matched for major histocompatibility antigens.

f) Antilymphocyte serum raised in horses, rabbits, burros, and goats has been extensively analyzed by in vitro assays and skin graft prolongation assays. As reported last year, the immunosuppression observed using 250 mg/kg of ALS in the skin graft prolongation testing regimen was correlated with assays of humoral antibody formation, rosette inhibition and immunoflourescent antibody. It has been found that animals can be tested using 50 mg/kg as a total dose without interfering with interpretation of the immunosuppressive properties. In each case, rosette inhibition correlated with the longer skin graft prolongation results, although other assays did not.

g) Six animals have received different antilymphocyte serum preparations in preparation for autologous bone marrow transplant studies. Only one animal receiving 50 mg per kilo 24 hours prior to marrow engraftment was successfully engrafted. Other animals receiving similar doses pre-transplant or during the post-transplant period (10 mg per kilo per dose on day 1, 3, 5, 7, etc.) did not survive the marrow transplant attempt. It has been interpreted that the hematopoietic toxicity of ALS must be overcome before we can successfully employ unaltered ALS in regimens such as these for human grafting studies. To this end the ALS preparations have been absorbed using peripheral blood granulocytes (B Cells) and have also been fractionated to Fab fragments of antibody. Each of these preparations has been tested by in vitro means to assay for hematopoietic toxicity using the in vitro marrow colony assay. These various antilymphocyte sera were found to have no loss of immunosuppressive properties when assayed in rosette inhibition. One of these preparations (absorbed with PMNS) has been incubated in vitro with donor bone marrow prior to transplantation to mismatched animals on six occasions. In each case there was failure of engraftment although large doses of marrow had been given. A second batch of similarly prepared ALS is being tested for hematopoietic toxicity with the in vitro marrow culture assay. The ALS fractionated into Fab fragments has not been used for grafting experiments, although it has been extensively tested in the mixed leukocyte culture assay and has been shown to have immunosuppressive properties.

h) Marrow transplant attempts have been performed in two gray collie dogs (cyclic neutropenia) in an attempt to restore normal hematopoiesis. These experiments are particularly aimed at defining whether this deficiency is a hematopoietic stem cell defect and therefore correctable through stem cell engraftment. In both cases, the animals succumbed to infection during the early post-irradiation therapy. In preparation for these gray collie transplant experiments, it was necessary to do mixed leukocyte culture analyses on litters of dogs possessing the gray collie. Several litters of gray collie dogs (affected gray

collies and normal dogs from the same litter) have been examined for DLA antigens and mixed leukocyte culture compatibility. These studies indicate that the majority of dogs having this hematologic defect are compatible with each other in the mixed leukocyte culture analysis (regardless of litter) and preliminary DL-A typing confirms this histocompatibility. Whether this can be attributed to the inbreeding that has occurred with collies or whether this represents an inherited hematologic defect related to other genetic variables such as histocompatibility antigens remains undefined. Several additional animals remain to be transplanted and from these studies it is hoped that an answer can be found with regard to the defect present in cyclic neutropenia.

i) Thirteen dogs with spontaneous neoplasms have been admitted for treatment following tumor biopsy, histologic evaluation and classification and complete hematologic examination. Each of these animals has received an extensive immunologic evaluation looking for reactivity in mixed leukocyte culture to random cells, tumor cells and mitogens including pokeweed and phytohemagglutinin. Dogs have also been followed for the number of spontaneous erythrocyte rosette forming cells in the peripheral blood (normal dogs having 15-30%). Animals have been treated with COP (cytoxin; vincristine and prednisone) or high dose L-asparaginase therapy. In each case there has been temporary irradication of tumor tissue with apparent remission. Several animals have gone into extended remission (6 wks. - 2 mos.) simultaneous with return of immunologic function to normal. It appears that dogs having persistently depressed immunologic assays failed to achieve long-term remissions. One animal received a frozen marrow autograft using tumor-free marrow removed during remission. This marrow was given 24 hours following 1,000 rads of total body gamma irradiation. The animal remained in clinical remission for four weeks post-transplant. It appears from these preliminary studies that dogs having tumors of lymphatic origin are responsive to chemotherapy regimens but will only evidence transient remissions. It is in this group of experimental animals that attempts to reduce the residual tumor cells with bone marrow transplantation, peripheral blood lymphocyte (stimulated and unstimulated) transfusions, and other forms of immunologic manipulation will be made.

j) In vitro lymphocyte suicide experiments have been continued using radioactive thymidine (high specific activity) and high specific activity uridine. In the best experiments it appears that at the most only 50% of reactive cells can be irradicated using this manipulation. Other investigators have suggested that suicide experiments could be used to irradicate a complete clone of donor cells which are responsive to histocompatibility antigen differences in a recipient. We have not found this to be satisfactory and have performed no transplants in dogs testing this postulate.

k) An assay for cellular immunity has been developed in the dog model and successfully used to follow alloimmunization to transplantation of skin and corneas and transfer of viable allogeneic cells. This assay has been employed to examine autologous tumor reactivity in animals bearing spontaneous neoplasms. The preliminary studies failed to identify reactivity against frozen tumor cells by mixed leukocyte culture or cellular cytotoxicity prior to chemotherapeutic regimens. This assay has also been adapted to measure cellular reactivity to dog epidermal cells. Initial experiments showed that epidermal cells can be chromium labeled and serve as targets to monitor allograft reactivity during skin graft rejection. It is anticipated that this modification of the cellular cytotoxicity can be used to monitor graft-versus-host reactivity in situations where recipient lymphocytes are not available for study. This assay has been adapted for human use and is currently being employed to monitor cellular reactivity following in vitro mixed leukocyte culture stimulation. This ability to monitor cellular reactivity in vitro may permit the identification of non-HL-A antigen histoincompatibility in otherwise histocompatible (HL-A matched) donor-recipient pairs. It is anticipated that this assay will be used to monitor tumor reactivity in patients with solid tumors.

Proposed Course of Project:

1) Continued evaluation of improved procurement and transfusion techniques for platelets and granulocytes. Of particular interest is the evaluation of donor selection using histocompatibility criteria and the relative effectiveness of leukocytes collected using various techniques.

2) Evaluation of leukocyte granulocyte antigen typing will be made in prospective leukocyte donors and compared to granulocyte types of recipient-patients.

3) Lymphocytes will be collected from normal donors with evidence of immunity to infectious agents using delayed hypersensitivity reactions (fungus and acid fast organisms) in order that transfer factor can be prepared and administered to patients with fevers of unknown origen. These studies will be performed in collaboration with investigators currently using transfer factor to treat immunologic deficiency states.

4) Continued HL-A and mixed leukocyte culture compatible bone marrow transplants on patients with refractory malignancy, leukemia or aplastic anemia.

5) Expanded autologous bone marrow transplantation studies using bone marrow harvested from patients without evidence of bone marrow disease.

6) Continued pre-clinical evaluation of factors essential for effective and reproducible bone marrow transplantation emphasizing particularly those factors related to the treatment of graft-versus-host disease.

7) Continued antitumor studies in animals bearing spontaneous neoplasms in order to examine immunotherapeutic manipulations and to identify the mechanisms of a successful therapeutic response using available in vitro assays.

Honors and Awards:

Invited Lectures:

Robert G. Graw, Jr., M.D.

1. Annual Meeting of the Swiss Hematological Society. Treatment of Leukemia and Aplastic Anemia with Histocompatible Allogeneic Bone Marrow Transplantation - A Review. Lausanne, Switzerland, June 8, 1972.

2. Special Conference at the 14th International Congress of Hematology. HL-A Identical Bone Marrow Transplantation - The Current Status of Bone Marrow Transplantation in the Treatment of Leukemia. Sao Paulo, Brazil. July 21, 1972.

3. Annual Meeting of the Michigan Association of Blood Banks. Normal Granulocyte Procurement for Transfusion Therapy. Detroit, Michigan. September 30, 1972.

4. International Symposium on Drugs and Hematologic Reactions. Bone Marrow Transplantation for the Therapy of Aplastic Anemia. Hahnemann Medical College. November 1, 1972.

5. Education Program, American Society of Hematology. Bone Marrow Transplantation in the Treatment of Aplastic Anemia. Hollywood, Florida, December 3, 1972.

6. Annual Meeting of the Cancer Nursing Association. Advances in the Treatment of Pediatric Malignancy - A Perspective. Northern Virginia Chapter, Alexandria, Virginia. January 25-26, 1973.

7. Visiting Professor of Medicine, University of Missouri School of Medicine, Presented seminar entitled "Current Trends in Bone Marrow Transplanta tion," and Medical Grand Rounds entitled "Granulocyte Transplantation Therapy." Columbia, Missouri. February 21-22, 1973.

8. Eighth Annual Clinical Cancer Conference: Perspectives in Cancer Chemotherapy and Bone Marrow Transplantation. Bone Marrow Transplantation in Acute Leukemia. San Francisco, California. March 16, 1973.

9. Hodgkin's Disease, Leukemia, and Lymphoma sponsored by the Cleveland Clinic Educational Foundation. Bone Marrow Transplantation - A Review of Current Status. Cleveland, Ohio. March 22, 1973.

Geoffrey P. Herzig, M. D.

1. International Symposium on Drugs and Hematologic Reactions. Leukocyte Transfusion Therapy for the Therapy of Aplastic Anemia. Hahnemann Medical College. November 1, 1972.

Ronald A. Yankee, M. D.

1. Yankee, R. A., Halterman, R. H. and Leventhal, B. G.: Response to single donor platelet transfusions and in vitro tests of alloimmunization. Transplantation Society, San Francisco, September 1972.

2. Yankee, R. A. and Graff, K.: Histocompatible Platelet Transfusions from Unrelated HL-A Matched Donors. Proceeding of the International Blood Transfusion Congress. Washington, D. C. August, 1972.

3. Yankee, R. A. and Leventhal, B. G.: Correlation of increase in MLC reactivity with response to platelet transfusion in specific donors. Amer. Soc. Hemat., Hollywood, Florida, December 6, 1972.

Publications:

1. Herzig, G. P., Root, R. K. and Graw, R.G., Jr.: Granulocyte Collection by Continuous-Flor Filtration Leukopheresis. Blood 39: 554-567, 1972.

2. Levine, A. S., Graw, R. G., Jr., and Young, R.: Management of infections in patients with leukemia and lymphome: Current concepts and experimental approaches. Seminars in Hematology 9: 141-179, 1972.

3. Graw, R. G., Jr., Yankee, R. A., Rogentine, G. N., Leventhal, B. G., Herzig, G. P., Halterman, R. H., Merritt, C. B., McGinniss, M. H., Krueger, G. R. F., Whang-Peng, J., Carolla, R. L., Gullion, D. S., Lippman, M. E., Gralnick, H. P., Berard, C. W., Terasaki, P. I., and Henderson, E. S.: Bone marrow transplantation from HL-A matched donors to patients with acute leukemia: Toxicity and anti-leukemic effect. Transplantation 14: 79-90, 1972.

4. Graw, R. G., Jr., Yankee, R. A., Leventhal, B. G., Rogentine, G. N., Herzig, G. P., Halterman, R. H., Merritt, C. G., Carolla, R. L., Alvegard, T. A., Bull, J. M., McGinniss, M. H., Krueger, G. R. F., Gullion, D. S., Lippman, M. E., Berard, C. W., Whang-Peng, J., Trapani, R. J., Terasaki, P. I., Steinberg, A. S., Gralnick, H. R., and Henderson, E. S.: Bone marrow transplantation in acute leukemia employing cyclophosphamide. Experimental Hematology 22: 118-125, 1972.

5. Halterman, R. H., Graw, R. G., Jr., Leventhal, B. G., Johnson, G., House, S., Krueger, G. R.: Reactive lymphocyte blastogenesis during graft-versus-host reaction. Transplantation 14: 271-274, 1972.

6. Halterman, R. H., Graw, R. G., Jr., Leventhal, B. G., and Fuccillo, D. A.: Immunocompetence following allogeneic bone marrow transplantation in man. Transplantation 14: 689-697, 1972.

7. Graw, R. G., Jr., Herzig, G. P., Perry, S., and Henderson, E. S.: Normal granulocyte transfusions: Treatment of septicemia due to gram negative bacteria. New Eng. J. of Med. 287: 367-371, 1971.

8. Rogentine, G. N., Jr., Rosenberg, E. B., Merritt, C. B., Rosenberg, S.A., Yankee, R. A., Graw, R. G.,Jr., Griep, P., Whang-Peng, J., and Fahey, J. L.: Antilymphocyte globulin as the sole immunosuppressant for bone marrow transplantation in aplastic anemia. Submitted for Publication.

9. Halterman, R. H., Graw, R. G., Jr., Bleyer, W. A., Johnson, G., Leventhal, B. G.: Adoptive immunity to leukemia. Submitted for Publication.

10. Graw, R. G., Jr.: HL-A identical bone marrow transplantation - The current status of bone marrow transplantation in the treatment of leukemia. Monograph Summary, 14th International Congress of Hematology, Sao Paulo, Brazil, 1972.

11. Graw, R. G., Jr. and Herzig, G.P.: Treatment of leukemia and aplastic anemia with histocompatible bone marrow transplantation - A review. Schweizerische Mediziniche Wochenschrift 102: 1573-1581, 1972.

12. Graw, R. G., Jr. and Yankee, R.A.: Symposium on clinical signs of blood disease. Principles of Hematologic Supportive Care. Medical Clinics of North America 57: 441-461, 1973.

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Serial No. NCI-6830
1. Pediatric Oncology Branch
2. Section on Infectious Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: 1) Studies on the Genetic Organization of the Oncogenic Papovavirus, SV40, Using Nondefective Adenovirus 2-SV40 Hybrid Viruses

2) Studies on Acute Infection, In Vivo Tumorigenesis, and In Vitro Transformation Induced by DNA and RNA Tumor Viruses: Viral and Host Components as Assayed by Biochemical and Biophysical Techniques

3) Studies on the Mechanism of Activation of RNA Tumor Viruses by Halogenated Pyrimidines

4) Infectious Complications of Malignancy: Natural History, Prevention, Diagnosis, and Therapy

Previous Serial Number: NCI-3407 and NCI-3416

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Other Investigators: Cephas Patch, Ph.D., Lowell E. Schnipper, M.D., James H. Breeden, M.D., Leo Orr, M.D., Yvonne Wewerka-Lutz, M.D., Dorothy Moore, M.S., Andrew M. Lewis, Jr., M.D., Wallace P. Rowe, M.D., Douglas Lowy, M.D., Michael N. Oxman, M.D., Myron Levin, M.D., Thomas J. Kelly, Jr., M.D., Ph.D., Sherman Weissman, M.D., Patrick Henry, M.D., Henry Westphal, Ph.D., Steven Baum, M.D., Robert E. Gallagher, M.D., George Khoury, M.D., Sisir Chattopadhyay, Ph.D., Natalie Teich, Ph.D., Stuart Siegel, M.D., John E. Bennett, M.D., Sheldon Wolff, M.D., Herbert Reynolds, M.D., Joel Myers, M.D., Donald Henson, M.D., James A. Rose, M.D.

Cooperating Units: Laboratory of Viral Diseases, NIAID; Research Division of Infectious Diseases, Boston Children's Hospital Medical Center and Harvard Medical School; Division of Hematology-Oncology, Department of Medicine, University of Missouri; Laboratory of Clinical Investigation, NIAID;

Laboratory of Pathology, NCI; Division of Hematology, Department of Medicine, Yale University; Division of Infectious Diseases, Department of Medicine, Albert Einstein College of Medicine; Section of Virology, Department of Microbiology, Johns Hopkins University; Laboratory of Tumor Cell Biology, NCI; Laboratory of Biology of Viruses, NIAID; Laboratory of Molecular Genetics, NICHD; Immunizations Branch, CDC (Atlanta); Division of Hematology, Los Angeles Children's Hospital.

Man Years:

Total: 28*
Professional: 18
Other: 10

*Does not include administrative, nursing, or paramedical services.

Project Description:

Objectives:

1) To study the genetic organization of SV40 by an analysis of the varying segments of the SV40 genome contained within adenovirus-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical and electron microscopic techniques to arrive at a genetic map of an animal tumor virus.

2) To identify and isolate that portion(s) of SV40 which relate most directly to the virus' oncogenicity, employing the previously noted adenovirus-SV40 hybrid viruses.

3) To analyze the behavior of DNA and RNA virus-transformed cells as a function of the molecular conformation of viral DNA within these cells, and as a function of events which may control viral genetic expression.

4) To analyze the behavior of permissive cells acutely infected by DNA and RNA viruses as a function of the molecular conformation of the viral nucleic acids and as a function of events which may control viral genetic expression.

5) To study the molecular mechanisms by which the human herpes viruses establish a latent state, as it relates to recurrent infection and to putative tumorigenicity. Such mechanisms will be approached with the techniques previously applied successfully to the smaller DNA tumor viruses.

6) To analyze the mechanism by which C-type murine leukemia virus is activated by the thymidine analogs, 5-iododeoxyuridine (IUdR) and 5-bromodeoxyuridine (BUdR).

7) To evaluate the utility and liabilities, separately and together, of various procedures designed to reduce the incidence of severe infection in patients with acute leukemia and in other compromised hosts; to evolve prophylactic techniques and equipment with greater utility than presently available.

8) To improve methods by which occult infectious agents may be detected and eradicated, e.g., latent herpes viruses, L-forms of bacteria, fastidious anaerobic bacteria, mycoplasma, and fungi.

9) To evaluate the human germ-free state, as it occurs in patients with leukemia, and to learn what the influence of this state is on normal physiologic and metabolic processes, and on the metabolism of cancer chemotherapeutic agents.

10) To improve our understanding of the natural history of unusual opportunistic infections, seen primarily in compromised hosts.

Methods Employed:

Standard biochemical and radioisotopic methods have been employed for the measurement of nucleic acid and protein synthesis. Procedures for the extraction of highly purified viral and cell nucleic acids have been further improved. New techniques for hybridization of viral and cellular nucleic acids have been developed, and modifications which permit more quantitative comparison of heterologous nucleotide sequences have been described. Viral DNA has been analyzed by equilibrium density centrifugation (neutral and alkaline), analytical ultracentrifugation, ethidium bromide banding of superhelical DNA, temperature-absorbance profiles, and electron microscopic techniques. Standard tissue culture techniques have been carried out, as well as plaque assays, complement-fixing assays, and immunofluorescent assays for viral antigens. Localization of SV40 genetic material in adenovirus-SV40 hybrid DNA molecules has been accomplished by electron microscopic examination of heteroduplexes formed with one strand of wild-type adenovirus DNA and one strand of hybrid virus DNA. Methods for separation of the strands of adenovirus DNA, using poly IG and isopycnic density separation, have been developed.

Procedures for fractionating the chromosomes of virus-transformed cells according to group-size have been improved, as have hybridization methods to determine which chromosomes contain integrated viral DNA (using in vitro synthesized complementary RNA).

The technique of hydroxyapatite chromatography has been employed to separate single- and double-stranded DNA. Gel electrophoresis has been utilized in an attempt to purify virus-specific proteins from infected and transformed cells. Viral DNA probes have been synthesized, utilizing the endogenous reverse transcriptase activity of murine leukemia viruses. A combined enzymatic, chromatographic, and nucleic acid hybridization approach has permitted nucleotide sequencing of the SV40 RNA transcripts corresponding to specific strands and areas of SV40 DNA.

Quantative evaluation of the efficacy of prophylactic techniques in compromised hosts is based upon comprehensive sampling of the patients and their environment for aerobic and anaerobic bacteria, fungi, mycoplasma, and viruses. The major prophylaxis undertaken is laminar air flow isolation-filtration and chronic bowel decontamination. Routine and special culture procedures are employed, as are serologic assays. Criteria for the diagnosis of infection are based on autopsy or biopsy results, cultures, x-rays, and clinical impression. Results are subjected to multiple statistical tests. In vivo and in vitro investigation of possible toxicity from prophylactic techniques is conducted. Routine and special techniques are applied to the isolation of occult agents, immunofluorescent and other serologic assays, and animal inoculation. Such laboratory procedures are correlated with morphologic and cultural results gathered from pathologic specimens. The limulus assay for circulating endotoxin has been modified and applied to immuno-suppressive patients.

Major Findings:

1. Nondefective Ad2-SV40 Hybrid Viruses: Biological Properties. Four new nondefective adenovirus 2 (Ad2)-simian virus 40 (SV40) hybrid viruses have been isolated. Although these viruses (designated $Ad2^{+}ND_2$, $Ad2^{+}ND_3$, $Ad2^{+}ND_4$, and $Ad2^{+}ND_5$) were clonal derivatives of the same Ad2-SV40 hybrid population, they differ significantly from each other and from the previously isolated nondefective hybrid, $Ad2^{+}ND_1$, in their biological properties or in the amount of SV40-specific ribonucleic acid induced during lytic infection.

Like $Ad2^{+}ND_1$, $Ad2^{+}ND_2$ and $Ad2^{+}ND_4$ pass serially in both human embryonic kidney (HEK) and primary African green monkey kidney cells. In contrast, $Ad2^{+}ND_3$ and $Ad2^{+}ND_5$ pass serially only in HEK cells. $Ad2^{+}ND_2$ is like $Ad2^{+}ND_1$ in that it induces the SV40 U antigen, but not SV40 T antigen; however, in contrast to the perinuclear SV40 antigen induced by $Ad2^{+}ND_1$, the SV40 antigen induced by $Ad2^{+}ND_2$ is located peripherally in the cytoplasm as well as in the perinuclear region of infected cells; $Ad2^{+}ND_2$ also induces the SV40 tumor-specific transplantation antigen (TSTA). $Ad2^{+}ND_4$ induces the SV40 T and U antigens and TSTA. $Ad2^{+}ND_3$ and $Ad2^{+}ND_5$ do not induce serologically detectable SV40 antigens and are distinguished from each other on the basis of the relative quantities of SV40-specific RNA which they induce. The induction of different SV40-specific functions suggests the incorporation of different segments of SV40 deoxyribonucleic acid within the genomes of the respective hybrid viruses.

2. Nondefective Hybrids: SV40 Content and Configuration. Certain biophysical characteristics of the DNA from each of the five nondefective adenovirus 2 (Ad2)-simian virus 40 (SV40) hybrid viruses ($Ad2^{+}ND_1$, $Ad2^{+}ND_2$, $Ad2^{+}ND_3$, $Ad2^{+}ND_4$, $Ad2^{+}ND_5$) have been determined. The guanine plus cytosine content varied from 55 to 57% and was not significantly different from that of nonhybrid Ad2 (56%), and the hybrid DNA molecules had mean molecular lengths which were similar to that of the standard, Ad2. The Ad2 and SV40 components of each hybrid were linked by alkali-resistant, presumably

covalent, bonds. The percentage of SV40 DNA in each hybrid virus was determined by hybridization with SV40 complementary RNA in a calibrated system. The results indicate that each hybrid virus DNA contains a different percentage of SV40 nucleotide sequences. The quantity of SV40 DNA present is a stable characteristic of each hybrid virus, and varies from less than 5% (in $Ad2^{+}ND_3$) to more than 30% (in $Ad2^{+}ND_4$) of the SV40 genome.

3. Nondefective Hybrids: SV40 RNA Species and Genetic Mapping of Early SV40 Functions. We have characterized the SV40 portions of these hybrids by relating the SV40-specific ribonucleic acid (RNA) sequences transcribed in cells infected with each hybrid virus to those transcribed in cells infected with each of the other hybrid viruses, and with SV40 itself. RNA-DNA hybridization-competition experiments indicate that the number of unique SV40 RNA sequences transcribed in infected cells is proportional to the size of the SV40 DNA segment contained within each hybrid and, in the case of the three hybrids which induce detectable SV40-specific antigens, to the number of SV40 antigens induced. Furthermore, the SV40-specific RNA sequences transcribed from any one of the hybrids are completely represented in the RNA transcribed from all other hybrids with longer SV40 segments. Thus, the SV40 DNA regions in the five hybrid viruses appear to contain some nucleotide sequences in common. The SV40-specific RNA transcribed from $Ad2^{+}ND_4$, the hybrid containing the largest SV40 segment, is qualitatively similar to the SV40-specific RNA transcribed early (i.e., prior to viral DNA replication) in SV40 lytic infection. Thus, it appears that no significant amount of late SV40 DNA is transcribed during infection by any of the five nondefective Ad2-SV40 hybrid viruses.

These data indicate that there are two possible arrangements for the three early functions on the segment of SV40 in ND_4: U, TSTA, T; or T, U, TSTA. Electron microscopic heteroduplex mapping has been employed to visualize the SV40 pieces in the five hybrids, and to specify their anatomic linkage in relation to wild-type SV40. In all of the hybrids, the SV40 insertion site is about 14% from one end of the molecule, the SV40 DNA pieces all overlap at one end, and the opposite end varies in length. These findings make the former arrangement for the three early genes of SV40 highly probable, i.e., U, TSTA, and T in linear order. Consequently, the isolation of these five nondefective hybrid viruses has now permitted the genetic mapping of the early region of SV40, i.e., that which appears to function in transformed cells.

4. Nondefective Hybrids: Pattern of SV40 Transcription. The SV40-specific RNA synthesized in cells lytically infected by the five nondefective hybrid viruses was reacted with separated strands of SV40 DNA (produced with hydroxyapatite chromatographic methods). It was found that the entire segment of SV40 DNA in each hybrid is transcribed, and that the transcript includes "anti-late" sequences and early sequences (- strand), but not late sequences (+ strand). This result supports the hypothesis that when SV40 DNA is in the linear form, as opposed to the native circular form, late information cannot be transcribed. This failure of late transcription (+ strand) is also found in cells transformed by SV40.

5. SV40 Transcription-Control Region in $Ad2^{+}ND_1$. The complementary DNA strands of the nondefective adenovirus 2 (Ad2)-simian virus 40 (SV40) hybrid virus, $Ad2^{+}ND_1$, were separated by isopycnic banding in a CsCl density gradient in the presence of synthetic polyribonucleotides. Separated strands were used in DNA-RNA hybridization reactions with RNA from cells productively infected by Ad2 or SV40, and with complementary SV40 RNA transcribed asymmetrically in vitro. About five times as much Ad2 RNA hybridized to the light strand of $Ad2^{+}ND_1$ as to the heavy strand. Complementary RNA and early SV40 RNA (RNA synthesized before viral DNA replication) had significant homology only with the light strand. Only half as much of a preparation of RNA synthesized before and after viral DNA replication (early-plus-late SV40 RNA) hybridized to the light strand as to the heavy strand. These results indicate that templates for both late and early SV40 RNA are present in $Ad2^{+}ND_1$. Therefore, the small SV40 segment within this virus (10-18% of the SV40 genome) must contain a transcription-control region. $Ad2^{+}ND_1$ should thus be useful in the selective study of transcription as it occurs in cells infected by the oncogenic virus SV40.

6. The Early Template of SV40 is Discontinuous. The genomes of five nondefective adenovirus 2 (Ad2)-simian virus 40 (SV40) hybrid viruses contain overlapping segments of the early region of wild-type SV40 DNA. The complementary strands of these hybrid genomes were separated by isopycnic banding in the presence of synthetic polyribonucleotides. Separated strands were used in DNA-RNA hybridization reactions with SV40-specific RNA synthesized before and after viral DNA replication (early-plus-late RNA). This RNA hybridized to both strands of each hybrid virus, indicating that templates for both late and early SV40 RNA are present in all five viruses. The relative amounts of these templates in each virus were determined from the distribution of hybridizable early-plus-late RNA, and a map of the early SV40 region was constructed on the basis of these data. The results provide evidence that the early template of SV40 is discontinuous, and smaller than currently believed. This evidence, if confirmed, raises important questions about the size and nature of the early transcription and protein products of this oncogenic virus.

7. Induction of Cellular DNA Synthesis by the Nondefective Hybrids. Induction of cell DNA synthesis by the Ad2-SV40 hybrid viruses was examined in monkey and hamster cells. It was not possible to discriminate the effects of the hybrid viruses from those of wild-type Ad2: Induction was inhibited by prototype and hybrids in monkey cells and stimulated by all viruses in hamster cells.

8. Transformation of Hamster Cells by the Nondefective Hybrids. As with the induction phenomenon, we have not yet been able to discriminate morphologic transformation induced by the hybrids from that induced by the prototype Ad2. Further experiments are underway to refine this important discrimination. It is of interest that most hybrid-transformed cells fail to synthetize SV40-RNA. Only $Ad2^{+}ND_4$ and one of three lines of $Ad2^{+}ND_2$-transformed cells are positive in this regard. All hybrid-transformed lines produce Ad2 RNA, however.

9. Ad2^{++}HEY and LEY. SV40 nucleotide sequences in the hybrid genomes and in cells infected with the adenovirus 2 (Ad2)-SV40 hybrids, Ad2^{++}HEY and Ad2^{++}LEY, were analyzed. These two variants of the Ad2-SV40 hybrid population differ significantly in their ability to yield SV40 virions. The variants induce SV40 T antigen with equal efficiency, but only the high efficiency yielder (Ad2^{++}HEY) induces detectable SV40 V antigen. In an attempt to understand the difference in the biological activity of these hybrids, we determined the content and character of SV40 nucleotide sequences in infected monkey cells and in the hybrid genomes. Hybridization-competition studies indicated that the low efficiency yielder (Ad2^{++}LEY) induces the full complement of late SV40 RNA sequences, but at a 10- to 100-fold lower concentration than does Ad2^{++}HEY. When viral DNA was selectively extracted from cells and analyzed in alkaline sucrose gradients, circular forms of SV40 were detected only following Ad2^{++}HEY infection. Electron microscopy study of heteroduplexes formed between hybrid DNA and Ad2 DNA indicated that the Ad2^{++}LEY genome contains 1.0 unit length of SV40 DNA inserted 7% from one end of the hybrid molecule and accompanied by an 11% Ad2 deletion. Ad2^{++}HEY molecules had 1.4 or 2.4 SV40 units inserted 28% from one end, with a 38% Ad2 deletion. These results suggest that inefficient yielding of SV40 by Ad2^{++}LEY is due to infrequent excision of an intact SV40 genome from the hybrid molecule. The importance of this HEY-LEY system lies in the fact that the configuration of SV40 and adeno-DNA within these hybrids may bear upon the relation of SV40 to host DNA in SV40-transformed cells; thus, the system might provide a clue as to which mechanisms are necessary to permit a tumor virus genome to be excised from its integrated state (excision presumably being associated with viral replication and cell lysis).

10. Viral Nucleic Acids in Transformed Cells Differing in Oncogenicity. The virus-specific RNA and DNA of two clonal lines of simian virus 40 (SV40)-transformed hamster heart cells differing in oncogenic potential were studied by nucleic acid hybridization techniques. Reconstruction experiments, with radiolabeled SV40 complementary RNA synthesized *in vitro* used as a probe, indicated 2.0 viral genome-equivalents of SV40-specific DNA per cell in the line with high oncogenic potential and 1.3 viral genome-equivalents per cell in the line with low oncogenic potential. Cells of the low oncogenicity line appeared to synthesize less than one-fourth as much SV40-specific RNA as did cells of the high oncogenicity line, but qualitative differences could not be demonstrated.

11. Activation of Murine Leukemia Viruses. In Dr. Rowe's laboratory, screening of various compounds had shown that the two thymidine analogs, 5-bromodeoxyuridine (BrdU) and 5-iododeoxyuridine (IdU), are by far the most efficient inducers of MuLV in virus-negative cell lines derived from AKR mouse embryos. Current investigations of the mechanism of virus induction by BrdU and IdU suggest that incorporation of either halogenated pyrimidine is a prerequisite to viral activation. This has been demonstrated by using isotopically labeled analog under a variety of conditions and correlating incorporation into DNA with viral activation; also, analog-treated cells have been found to have their rate of induction enhanced when treated with visible light, ultraviolet light, or x-irradiation.

12. Use of Viral DNA Probes Made with Reverse Transcriptase. Isotopically labeled single-stranded viral DNA probes have been synthesized from purified AKR and Kirsten MuLV utilizing the virion associated endogenous reverse transcriptase in the presence of actinomycin D (method of E. Scolnick). Utilizing this probe, DNA reassociation kinetics with a variety of cellular DNAs have been carried out as described originally by Britten and Kohne. Significant differences in hybridization have been noted between DNA from uninfected NIH (low leukemic) and AKR (high leukemic) mouse embryo cells. These differences have been noted with both AKR and Kirsten probes. A cloned wild mouse embryo cell line from our laboratory which has an unusually high degree of sensitivity to non-tissue culture adapted MuLV has been found to contain the smallest amount of hybridizable material of the various mouse cells tested. The nucleic acid hybridization studies confirm the reports of others that cellular DNA contains sequences which are complementary to viral nucleic acids. These findings, when combined with results from many other laboratories using a variety of approaches, suggest that endogenous RNA virus genomes are present in cells as DNA, probably integrated into the host genome. Furthermore, our studies suggest that mouse strains differ in the number of copies of virus genomes per cell, a situation which differs from the results reported with chicken strains.

13. Lack of Sequence Homology. No significant hybridization was detected of deoxyribonucleic acid from simian virus 40 or polyoma virus and 70S ribonucleic acid from avian myeloblastosis virus, murine leukemia virus (Rauscher), murine sarcoma virus (Kirsten), RD-114B, simian sarcoma virus-1 or Mason-Pfizer virus.

14. Protected Environments and Prophylactic Antibiotics. To reduce the frequency of infection in acute leukemia, we employed isolation and air-filtration facilities ("protected environment") and a prophylactic regimen that included oral nonabsorbable antibiotics. Eighty-eight randomized patients received identical remission-induction chemotherapy within one of three groups: protected environment combined with the prophylactic regimen (Group 1); oral nonabsorbable antibiotics alone (Group 2); and neither isolation nor prophylaxis (Group 3). The groups were comparable in factors that might influence the course of leukemia and susceptibility to infection. Environmental maneuvers were effective in reducing the potential inoculum of ambient micro-organisms. Patients in Group 1 had one-half as many severe infections as those in Groups 2 and 3. Whereas approximately one-fourth of the patients in Groups 2 and 3 died of infection while on study, none in Group 1 died for that reason. Despite fewer infections in Group 1, no intergroup differences were found in remission rate or duration. The ultimate benefits of reduced infectious morbidity in leukemia may depend on further advances in chemotherapy.

15. Cytomegalovirus Infections During Acute Childhood Leukemia. Cytomegalovirus (CMV) infections were studied longitudinally in 88 leukemic children; 24 patients had cultural evidence of CMV infection at some point. There appeared to be a good correlation between excretion of virus (in urine and/or

throat) and presence of complement-fixing (CF) and indirect-hemagglutination (IHA) antibody; however, a few patients had detectable antibody without excretion of virus and vice versa. Compared with patients not excreting virus, patients shedding CMV had significantly more episodes of pneumonitis and fever with rash but did not have more episodes of hepatitis, fever of unknown origin, or upper respiratory-tract infections. Clinical syndromes attributed to CMV correlated with fourfold rises in CMV antibody titer only during hematologic remission. However, dissemination of CMV occurred during hematologic relapse as well as during remission. Three patients who died after fourfold rises in CMV antibody had postmortem evidence of dissemination. Excretion of virus did not correlate with hematologic relapse, appearance of symptoms, antibody levels, or lymphocyte transformation against CMV. These data suggest that the urine and throat should be repeatedly cultured to detect infection with CMV. Moreover, to diagnose dissemination of CMV infection, it is necessary to demonstrate a fourfold rise in antibody to the virus.

16. The Doctor-Patient Relationship: A Seminar for Oncology Fellows. In an attempt to deal with the anxiety aroused in young physicians who must care for terminal patients, and with the often inappropriate defenses employed in response to this anxiety, we organized a practical teaching seminar which utilized the principles of preventive and social psychiatry. Six to ten Oncology Fellows met weekly with a psychiatrist and a senior attending physician for approximately 5 months. The major initial defenses of the Fellows were identified as anger and denial. The most severe problems discussed were the formation of dependency relationships between doctors and patients, the patient's denial of illness, the phenomenon of social death, and the physician's identification with his patient. The latter problem often appears to be a productive one, since it encourages the physician to confront the basic issues of his own life. When this confrontation had been examined, most Oncology Fellows became increasingly effective with their patients.

17. Super-intensive Chemotherapy of Acute Myelocytic Leukemia in a Protected Environment. During the past year, ten adults have been given induction therapy with arabinoside cytosine + 6-thioguanine while randomized to a further study of laminar-air-flow and prophylactic antibiotics. Five patients received therapy to the point of bone marrow aplasia and for up to one week thereafter; two received therapy just to the point of aplasia; and three received therapy just to the point of aplasia while on the open ward without antimicrobial intervention (control). In the first group, four patients died during induction therapy and one remitted. Of the four deaths, three were associated with severe infection. In the second group, one died and one remitted; the one death was associated with severe infection. In the third group, there were two deaths and one remission, and one of the deaths was associated with severe infection. Although the sample sizes are far too small for statistical analysis, the trends suggest that the comprehensive prophylactic routine has no utility in this particular induction therapy schedule for adult AML.

18. Pseudomonas Vaccination. Fifteen leukemic patients have been vaccinated with a polyvalent pseudomonas vaccine while in remission. All but one have had antibody titers in response to the immunization of greater than 1:200, and these titers have persisted through the time of this report. The first few patients had moderate to severe toxicity, but this has not been a problem in subsequent patients who have been treated simultaneously with a corticosteroid. None of these patients has had a Pseudomonas infection since vaccination, and significantly, none has shown colonization with this organism.

19. Limulus Assay for Endotoxin. Ninety-three patients with leukemia, receiving chemotherapy, have been studied for the presence of endotoxin during febrile episodes. Of this group, twenty-three had positive blood cultures, 19 of whom had a simultaneously positive limulus assay. One additional patient had a positive limulus test but a negative culture. It thus appears that the test demonstrates a correlation with culture results, but additional data is required to determine the test's reliability, and to enhance this reliability with further development of the assay.

20. Zoster Immune Globulin for Varicella Prophylaxis. Eighteen children with leukemia, who had no history of varicella, were given either zoster immune globulin or immune serum globulin (pooled) within 72 hours of exposure to varicella. None in either group developed varicella, and it is likely that this low attack rate reflects the fact that exposure criteria were not sufficiently stringent.

21. Phototherapy Treatment of Recurrent Herpes Simplex Lesions. Fifteen patients with recurrent simplex received phototherapy treatment of vessicles. Nine patients died of unrelated causes within 3 months; the six remaining appear to have a 25-50% reduction in the rate of simplex recurrence, but the numbers are too small to permit statistical analysis at this time.

Proposed Course:

1. Studies will continue on hamster cells transformed by the five non-defective Ad2-SV40 hybrid viruses presently isolated and characterized. We will attempt to account for variation in the synthesis of SV40-specific RNA in the various transformed lines and in cells cloned from the lines: a determination will be made of the number of genome-equivalents of SV40 and of Ad2 DNA, and the pattern of SV40 and of Ad2 transcription will be analyzed using separated strands of each virus' DNA. The goal of the project is to determine if, in fact, a given segment of the SV40 genome is responsible for oncogenicity or contributes to it--a goal which can now be approached using the precisely defined and mapped SV40 regions previously discussed. The planned experiments will also speak to the molecular biology of transformation by the human wild-type adenovirus 2.

2. The evidence that the early region of SV40 is discontinuous (see Findings) will be pursued in an electron microscopy experiment. A combination of enzyme digestion and heteroduplexing--using separated SV40 strands--should

permit a definitive statement as to the topography of transcription control in this important region of the SV40 genome.

3. The topographical analysis of SV40 templates will be continued using a defective Ad-SV40 hybrid which contains 75% of the SV40 genome ($E46^+$) and using linear wild-type SV40 molecules (created with R_1 restriction enzyme). These experiments should complete the assignment of early and late templates within the entire SV40 genome.

4. A number of experiments are planned which employ the technique of Ad2 strand separation already described. These include: (a) Nucleotide sequencing of the SV40 RNA which hybridizes to separated strands of the nondefective hybrid DNAs. This should provide further detail on the genetic map of SV40. (b) We will determine if transcription of the Ad portion of the hybrid genomes in acutely infected cells shows any differences re extent and strand orientation vis-a-vis transcription of wild-type Ad2. (c) It is known that coinfection with SV40 enhances the ability of adenovirus 2 to replicate in monkey cells (ordinarily nonpermissive for Ad2). The mechanism of enhancement might involve alterations of Ad2 transcription, and this will be approached using separated Ad2 DNA strands. (d) Separated strands will also be used in a collaboration with Dr. Henry Westphal, the goal of which is to perform a genetic mapping analysis on adenovirus, as has now been performed with SV40. (e) SV40-specific RNA from a variety of SV40-transformed cells will be reacted with separated hybrid DNA strands to learn if there is a limited area of SV40 transcription (from a single strand) detectable in all transformed cells. (f) About 20% of the Ad2 DNA from acutely infected cells is single-stranded. This DNA is assumed to be a strand of parental DNA whose complementary strand has replicated but which itself has not yet done so. We shall challenge this single-stranded DNA with separated strands of Ad2 DNA to determine which the single-stranded material is complementary to--one or both strands.

5. In collaboration with Dr. Lewis (LVD, NIAID), an attempt will be made to produce new recombinants of SV40 and Ad2 which contain deletions in the various segments of the SV40 genome already isolated in the present hybrids. We shall carry out biochemical characterization of the new recombinants to determine the effect of altering various regions of SV40 DNA and RNA on the induction of SV40 biologic functions.

6. Long-term studies on the separation of chromosomes from virus-transformed cells, using differential centrifugation, will continue. The goal is to isolate and identify integration site(s). Other long-term studies on the proteins induced in infected and transformed cells by SV40 will also continue, the goal being the characterization of these proteins, and in particular, whether "viral-specific" antigens are partially coded by the host as a result of integration ("polycistronic messenger").

7. Studies on the mechanism of IdU and BrdU activation will be extended. We plan to determine whether analog incorporation during a critical portion of

the cell cycle is necessary and whether critical sites of cellular DNA must be analog-substituted for viral activation. In addition, nucleic acid hybridization studies will be carried out to determine the kinetics of viral-specific RNA production during IdU virus induction. Finally, it will be determined if IdU mediated induction is associated with viral-specific sequences going from high to low molecular weight DNA.

Further definition of the viral DNA probes will permit a more quantitative analysis of the number of viral genome copies in different mouse cells, as determined by DNA reassociation kinetics. This investigation will be coordinated with the animal genetics experiments of Rowe and Hartley to determine if the virus-inducing genes Akv-1 and Akv-2 represent structural viral genes.

We shall also attempt to visualize the surfaces of activated cells with the scanning electron microscope.

The anatomic sites of various antigens induced by both RNA and DNA viruses will also be examined, using the technique of ferritin-tagged antibody as visualized in the transmission electron microscope.

8. Collaborative experiments on herpes virus transcription will continue, using many of the same techniques described or modified for SV40 and adenovirus 2. Of special interest are potential differences in viral transcription between cells of Burkitt lymphoma and infectious mononucleosis.

9. Laminar air-flow studies have been suspended because of an acute nursing shortage; it is hoped that such studies can be resumed in the summer of 1973, following removal of the laminar flow units to a new NCI infectious disease unit within the Clinical Center. When resumed, it is planned to apply the prophylactic regimes to pediatric and adult solid tumors, aplastic anemia, and bone marrow transplantation, and to determine the utility of this antimicrobial approach in improving survival in these various compromised hosts. New approaches to the prevention of infection involve animal gnotobiology experiments, and are being pursued under a contract with the University of Notre Dame (Dr. M. Pollard). Although the NCI intramural study of laminar air-flow in adult leukemia will be terminated because of the transfer of these patients to other NCI units, it is hoped that the data already gathered on super-intensive cytosine-6TG can be used to augment results being developed under contract to the M. D. Anderson Hospital (Dr. G. Bodey).

10. Studies on the polyvalent pseudomonas vaccine in leukemic patients will continue, thus permitting both an increased number of vaccinated patients and an increased clinical observation time for those already vaccinated. We shall also determine if the antibodies induced by this vaccine pass the respiratory epithelial barrier and/or are secreted into the gastrointestinal tract.

11. The Zoster-immune globulin study will also continue, with more stringent criteria for exposure having been recently instituted. The present dosage of

ZIG will be compared with an increased dosage of ISG, so that the maximum amount of ISG which can be administered is compared with a small and putatively effective amount of ZIG.

12. The limulus study will end with another 10-20 patients, but further studies are planned in which a more sensitive limulus lysate will be employed. We shall also undertake a serial study of endotoxemia as it is modified by successful antibiotic therapy.

13. The H. simplex photoinactivation study will also continue, to permit a statistically significant number of patients and a significant observation time.

14. We shall initiate a very extensive review of Candidiasis in all cancer patients seen at the NIH in the past decade (in collaboration with the Medicine Branch). This illne-s is the most common fungal complication of malignancy, yet disseminated disease can rarely be diagnosed before death. Since serologic assays and cultures are rarely helpful, if at all, an extensive clinico-pathologic correlation and cataloging of presenting syndromes may be the only way to improve diagnosis and therapy.

15. In collaboration with the Baltimore Cancer Research Center, we shall undertake a controlled trial in granulocytopenic, septic patients of (a) Tobramycin + Methicillin versus (b) Carbenicillin, Keflin, and Tobramycin.

Honors and Awards:

1. Named "Distinguished Alumnus" (annual award), The Chicago Medical School, October 1972 (Levine).

2. Editorial on recent work from this Laboratory: "Simian Virus 40 Transcriptional Control," Nature 240: 440-441, 1972.

3. Siegel, S. E., Kelly, T. J., Jr., Patch, C. T., Lewis, A. M., Jr., and Levine, A. S.: SV40 Nucleotide Sequences in the Hybrid Genomes and in Cells Infected with the Adenovirus 2-SV40 Hybrids, $Ad2^{++}HEY$ and $Ad2^{++}LEY$. Presented at the meetings of the American Society for Microbiology, Miami, May 1973.

4. Schnipper, L. S.: Studies in Molecular Virology. Presented at the Kellog Summer Institute, Fisk University, August 2-3, 1972.

5. Levine, A. S.: Germ-free Biology and the Patient with Malignant Disease. Clinical and Preclinical Studies. Presentation and Session Chairman, NCI Contractor's Meeting, Annapolis, October 1972.

6. Levine, A. A.: Protected Environment and Prophylactic Antibiotics. Seminar presented to the Department of Clinical Pathology, Clinical Center, NIH, April 1972.

7. Levine, A. S.: Laminar Air-Flow Studies--A Review. Seminar presented to the Laboratory of Clinical Investigations, NIAID, NIH, November 1972.

8. Levine, A. S.: Concepts and Approaches in the Management of Opportunistic Infections. Seminar presented at the Weekly Hematology Conferences, Department of Medicine, Georgetown University School of Medicine, March 1973.

9. Levine, A. S.: Infections in Patients with Cancer and Preventive Techniques. Seminar presented to the Hematology Branch, NCI, February 1973.

10. Levine, A. S.: Clinical Microbiology and Leukemia. Lecture presented at the Children's Cancer Research Foundation (Jimmy Fund), Harvard Medical School, February 1973.

11. Levine, A. S.: Studies on the Nondefective Adenovirus 2-SV40 Hybrid Viruses. Presented at the NCI (Division of Cancer Treatment) Review Meeting, Annapolis, June 1972.

12. Levine, A. S.: Biochemistry and Biophysics of the Defective and Nondefective Adenovirus-SV40 Hybrids, with Emphasis on Mechanisms. Seminar presented to the Laboratory of Biology of Viruses, NIAID, December 1972.

13. Levine, A. S.: The Use of the Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses in the Genetic Analysis of SV40. Lecture presented to the M. D. Anderson Hospital, Houston, January 1973.

14. Levine, A. S.: Genetic Mapping of an Animal Tumor Virus. Presented to the NIH Scientific Directorate, February 1973.

Publications:

1. Lewis, A. M., Jr., Levine, A. S., Crumpacker, C., Levin, M., Samaha, R., and Henry, P.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. V. Isolation of Additional Hybrids Which Differ in Their Simian Virus 40-Specific Biological Properties. J. Virology, in press (May 1973).

2. Henry, P. H., Schnipper, L. E., Samaha, R. J., Crumpacker, C. S., Lewis, A. M., Jr., and Levine, A. S.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. VI. Characterization of the Deoxyribonucleic Acid from Five Nondefective Hybrid Viruses. J. Virology, in press (May 1973).

3. Levine, A. S., Levin, M. J., Oxman, M. N., and Lewis, A. M., Jr.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. VII. Characterization of the Simian Virus 40 Ribonucleic Acid Species Induced by Five Nondefective Hybrid Viruses. J. Virology, in press (May 1973).

4. Patch, C. T., Lewis, A. M., Jr., and Levine, A. S.: Evidence for an SV40 Strand Selection Region in the Nondefective Adenovirus 2-Simian Virus 40 Hybrid Virus, $Ad2^{+}ND_{1}$. Proc. Nat. Acad. Sci. U.S. 69: 3375-3379, 1972.

5. Siegel, S., and Levine, A. S.: Virus-Specific Nucleic Acids in SV40-Transformed Hamster Cell Clones Varying in Oncogenic Potential. J. Nat. Cancer Inst. 49: 1667-1673, 1972.

6. Gallagher, R., Levine, A. S., Gillespie, D., and Gallo, R. C.: Lack of Sequence Homology Between the 70S Ribonucleic Acid of Various Ribonucleic Acid Tumor Viruses and the Deoxyribonucleic Acids of Simian Virus 40 or Polyoma. J. Virology, in press (June 1973).

7. Henry, P. H., Levin, M. J., and Levine, A. S.: Composition and Biochemistry of Lymphocytes and Plasma Cells (Chapter 91). In Hematology, Williams, W. J., Beutler, E., Erslev, A. J., and Rundles, R. W. (eds.). Blakiston, McGraw-Hill Publishers, 1972, pp. 782-791.

8. Levine, A. S., Graw, R. G., and Young, R. C.: Management of Infections in Patients with Leukemia and Lymphoma: Current Concepts and Experimental Approaches. Seminars in Hematology 9: 141-179, 1972.

9. Henson, D., Siegel, S., Fucillo, D., Mathew, E., and Levine, A. S.: Cytomegalovirus Infections During Acute Childhood Leukemia. J. Infect. Dis. 126: 469-481, 1972.

10. Levine, A. S. Siegel, S. E., Schreiber, A. D., Hauser, J., Preisler, H., Goldstein, I. M., Seidler, F., Simon, R., Perry, S., Bennett, J. E., and Henderson, E. S.: Protected Environments and Prophylactic Antibiotics: A Prospective Controlled Study of Their Utility in the Therapy of Acute Leukemia. New Eng. J. Med. 288: 477-483, 1973.

11. Levine, A. S.: Germ-free Biology and the Patient with Malignant Disease. Clinical and Preclinical Studies. Cancer Chemotherapy Rep., in press.

12. Levine, A. S., and Schimoff, S.: Antimicrobial Therapy in the Compromised Host (Immunologic Deficiency States, Alcoholism, Poisoning, and Malignancy), (Chapter 42). In Antimicrobial Therapy, Kagan, B. (ed.). Saunders Publishers, in press.

13. Artiss, K. L., and Levine, A. S.: The Doctor-Patient Relationship in Severe Illness: A Seminar for Oncology Fellows. New. Eng. J. Med., in press.

Serial Number NCI 3024
1. NCI-VA Medical Oncology Branch
2. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Chemotherapy of Cancer

Previous Serial Number: 3024

Principal Investigator: Oleg S. Selawry

Other Investigators: Martin H. Cohen, Patrick J. Creaven, Heine H. Hansen, Suryabala Kanhouwa, Mary J. Matthews, Aron Primack, Marc Straus, Larry Allen

Cooperating Units: The NCI-VA Medical Oncology Branch represents a joint effort of the National Cancer Institute and the Veterans Administration. Funds are provided by the National Cancer Institute, administered by the Veterans Administration Central Office. Facilities are provided by the Veterans Administration Hospital in Washington, D.C.

Man Years:

Total:	71 3/4
Professional:	13 3/4
Other:	58

Project Description:

Objectives:

1. Improvement of treatment for patients with bronchogenic carcinoma.
   a. Intramural program
   b. Extramural program

2. Introduction of new anticancer agents into clinical use and pharmacology of new antitumor agents.

3. Improved use of chemotherapeutic agents of established value.

1. Patients with advanced bronchogenic carcinoma were random allocated to either a two drug combination or a three drug combination. Patients with small cell carcinoma responded significantly longer to the three drug combination (CCNU, cyclophosphamide, methotrexate) than to the two drug combination (cyclophosphamide, methotrexate). The doubling of the median time to progressive disease from three months to six months led to the design of a new study for intensive chemotherapy plus radiotherapy, including radiotherapy to the brain "sanctuary." Patients are sub-randomized to intensive chemotherapy in the protective environment of Laminar Air Flow rooms. Patients with adenocarcinoma experienced a doubling of survival time from four to eight months on the three drug combination. These data are not yet significant at the $p = .05$ level; the study is continued. (Drs. Hansen, Selawry, Cohen, Creaven, Matthews, Andrews, Simon)

2. A multidisciplinary Working Party for Therapy of Lung Cancer was established and funded by contract as of April, 1973. The party established criteria for study design and evaluation of response and initiated three interrelated studies geared toward maximal cell kill and, hopefully, eradication of small cell carcinoma. Activation of studies for surgical adjuvant chemotherapy, radiotherapy and for combination chemotherapy of epidermoid carcinoma is imminent. Studies for therapy of adenocarcinoma and large cell carcinoma are expected to be operative by May, 1973. (Drs. Selawry, Hansen, Matthews, Andrews, Straus)

3. An International Workshop for Therapy of Lung Cancer was held at Airlie, Virginia in October, 1972 and led to major agreements on microscopical classification criteria for response and on study design. The results of agreement and of therapeutic studies are in press in Cancer Chemotherapy Reports. The Medical Oncology Branch accepted responsibility for worldwide exchange of information, in cooperation with the IUCC. (Drs. Selawry, Hansen, Matthews, Primack, Andrews)

4. Four new chemotherapeutic agents underwent initial clinical trial (Phase I), including isophosphamide (NSC 109724), bis-diosopiperazinyl propane (NSC 129943), epipodophyllotoxin ethylidene glucoside (NSC 141540) and thalicarpine (NSC 68075), including studies on mode of action (thalicarpine) and drug metabolism (isophosphamide). (Drs. Creaven, Allen, Cohen, Hansen, Newman, Chisholm)

5. It was noted that alkylating activity in the urine of patients on isophosphamide is not affected by acetylcysteine. Thus, bladder irrigation with this compound is probably useless. (Drs. Creaven, Allen, Cohen)

6. Pharmacologic studies with methotrexate showed only negligible biliary excretion in a patient with biliary fistula. (Drs. Creaven, Hansen)

7. The effect of BCNU against L1210 was markedly enhanced by combination with non-myelosuppressive agents such as retinoic acid, caffeine, and chlorpromazine. A Phase I study of CCNU + caffeine + chlorpromazine failed to show increased toxicity. A Phase II study will be advanced by ECOG. (Dr. Cohen)

8. Dr. Marc Straus was appointed to head the Cell Kinetics Laboratory.

9. Dr. Aron Primack was appointed to head the newly established Marker Substance Laboratory.

10. The scientific work of the Branch is reflected in 32 papers, 15 abstracts, and 52 outside lectures and seminars given by the staff.

Detailed Reports:

1. Improvement of Treatment for Patients with Bronchogenic Carcinoma

1.1 Staging and Quantitation of Response

1.11 Fiberoptic Bronchoscopy (V 5XX LA) Martin H. Cohen

From July 1, 1972 to March 15, 1973, 81 bronchoscopies were done. The primary purpose of the procedure has been two-fold: 1) to obtain tissue from patients to determine the histologic type of their bronchogenic carcinoma and 2) to provide another parameter to determine response to chemotherapy and to determine disease progression at the earliest possible time. The use of the fiberscope has proved successful for both of these purposes. The large majority of patients have had tumors accessible for biopsy, the exception being patients with adenocarcinoma of the lung. In those patients whose tumors were not visible, bronchial washings for cytology frequently revealed malignant cells. With regard to the second point, cytologies of bronchial washings are being done to determine the effects of chemotherapy on cytologic appearance of tumor cells. For patients with small cell carcinoma, tumor cells are also being studied with the electron microscope to determine ultrastructural detail both before and during chemotherapy.

1.12 Sputum Cytologies (V 5XX LA) Martin H. Cohen

In conjunction with the nursing staff and the Pulmonary Physiotherapy Department of the VA Hospital, all patients with bronchogenic carcinoma admitted to the Hospital undergo serial sputum collections for cytology. This will provide information on the value of sputum cytology for evaluation of tumor response (and progressive disease), on the role of various techniques including nebulization and chest physiotherapy on the yields of positive cytology, and will also provide material to determine the effects of chemotherapy on the cytologic appearance of tumor cells.

1.13 Bone Marrow Aspiration and Needle Biopsy (V 5XX LA) Martin H. Cohen, Heine H. Hansen, and Brenda Shelton

The Hematology Laboratory, over the period April 1, 1972 to March 31, 1973, has continued the study of bone marrow involve-

ment of bronchogenic carcinoma by evaluating bone marrow aspirations, biopsies, and bone marrow touch preparations on all new patients with this diagnosis. In addition, other patients on the Service have had bone marrow studies where clinically indicated. Thus, over a one-year period the number of examinations done were:

bone marrow aspirates	260
bone marrow biopsies	210
touch preps of biopsies	210
differential counts of bone marrow aspirates and peripheral smears	560

1.14 Fiberoptic Peritoneoscopy (V 5XX LA) Heine H. Hansen, Reginald Chisholm, and Stephen Newman

From April 1, 1972 to March 31, 1973, 36 peritoneoscopies were done. The primary aims of the procedure are to 1) directly view the surface of the liver and 2) obtain biopsies of suspicious areas under direct vision. The procedure was done on patients on the ST 53 Protocol for Treatment of Bronchogenic Carcinoma. Preliminary analysis indicates that the procedure is more useful in patients with small cell carcinoma and adenocarcinoma of the lung. Our data imply that liver scan, liver chemistries, and liver size are insufficient parameters for diagnosis of liver metastases, unless proven by biopsy. Biopsies are positive in about 15-20% of these patients with extensive disease, even in the presence of negative liver scans and normal liver function studies. Electron microscopic studies are now being done on biopsies from patients with small cell carcinoma.

1.15 Lumbar Puncture for Cytology and LDH - Isoenzyme Determination (V 5XX LA) Martin H. Cohen

Cerebrospinal fluids: As part of a prospective study to determine the frequency on central nervous system involvement in bronchogenic carcinoma evaluated by cytologic smears prepared by the cytocentrifuge and in order to follow meningeal leukemia in several patients with AML, 120 cytocentrifuge preparations were prepared and evaluated. Cell counts of CSF fluid have been done serially in 12 patients.

Cytologic examination of other body fluids: Cytocentrifuge preparations were evaluated for 12 patients with malignant cells in pleural fluid and for two patients with cells in ascitic fluid.

LDH isoenzymes: In an attempt to determine the pattern of LDH isoenzymes distribution in blood and CSF in patients with bronchogenic carcinoma, a total of 124 specimens of either serum of CSF were collected. The data will be analyzed shortly.

1.16 Development of Markers (V 5XX LA) Aron Primack

Incidence of "biological markers," both hormonal and non-hormonal, in cancer will be studied. The work will be divided into two sections:

1) In vivo: Serum and urine of patients with bronchogenic carcinoma will be analyzed for qualitative and quantitative presence of hormones (including HCG with its alpha and beta subunits, ACTH, calcitonin, and parathyroid hormone when its analysis becomes perfected) as well as non-hormonal compounds (including methylated bases, polyamines, alpha fetoprotein, carcinoembryonic antigen). These "biochemical markers" will be followed routinely during the patients' course to determine the correlation between the presence and amount of these compounds and disease activity.

2) In vitro: Cell lines of human bronchogenic carcinoma have been grown successfully in the past. One such line, which produced several abnormal hormones, is available for our use and is planned to determine the effects of growth, drugs, and environment on the production of these hormones.

1.17 Other Procedures (V 5XX LA) Martin H. Cohen

Liver touch preps 7
Lymph node aspirates 12
Blood drawing and centrifugation 7500 samples

1.18 Microscopical Slide Review of All Patients Assumed to Have Lung Cancer (-- 8XX LA) Mary J. Matthews and Suryabala Kanhouwa

Microscopic slide review of over 80 new patients with or suspected of having lung cancer was done before patients were accepted for treatment and/or assignment to protocol. Evaluation was made of extent of disease and cell type.

1.19 Specimen Review and Interpretation (-- 8XX LA) Mary J. Matthews and Suryabala Kanhouwa

During the year, cell blocks, smears, and nucleopore filter preparations of over 2200 sputum specimens, bronchial washings, and other body fluids were interpreted. Approximately 200 surgical specimens were interpreted or reviewed.
A study for the evaluation of pulmonary cytology for its reliability in diagnosis of cell type is in progress. A program is being developed for the evaluation and improvement of diagnostic criteria for lung cancer by electron microscopy, utilizing surgical, biopsy, and autopsy material.

1.110 Autopsies (-- 8XX LA) Mary J. Matthews and Suryabala Kanhouwa

Thirty autopsies were performed on patients who had been on lung cancer protocols. An autopsy protocol, devised by the Pathology Committee of the Working Party for Therapy of Lung Cancer, was utilized and evaluated, including estimation of lethal tumor cell burden at primary and metastatic sites, and validity of clinical staging. Microscopic slides of over 200 autopsies of patients who died with lung cancer were interpreted or reviewed. A review of all lung cancer autopsies in the Washington VA Hospital for the past ten years has begun. The review will check the

reliability and consistency of clinical diagnosis and staging and will utilize the WP-L lung classification, a slightly modified version of the WHO classification of lung tumors, published in 1967 by Kreyberg et al.

1.2 Therapy

1.21 Intensive Therapy of Patients with Primary Lung Cancer (VI 6X1 LA) Study ST 53: Heine H. Hansen, Oleg S. Selawry, and Staff with participation of Dr. David Carr, Mayo Clinic, and Dr. Rossall Sealy, University of Capetown

An uncontrolled study (ST 42), publication reference 13, had shown encouraging survival of patients with regional spread of lung cancer, receiving radiotherapy plus chemotherapy with cyclophosphamide + methotrexate. The current study was therefore initiated to: 1) compare radiotherapy alone and radiotherapy plus adjuvant chemotherapy in patients with regional spread of lung cancer, and 2) compare "standard" chemotherapy including two drugs (CTX + MTX) with a three drug combination (CTX + MTX + CCNU).

The better arm (if any) was to be used as standard for future protocols. A total of 339 patients were included and evaluated as of January 5, 1973, including 182 patients from the Medical Oncology Branch.

The distribution of patients by cell type (Table 1) shows an unusually high proportion of patients with bronchogenic small cell carcinoma and adenocarcinoma. This reflects the conceptual interest in these two tumor types because of their unusual potential for response.

Results:

1. No superiority was noted for adjuvant chemotherapy, as measured by survival.
2. The three drug combination was significantly superior to the two drug combination in small cell carcinoma (doubling of time from onset of chemotherapy to occurrence of progressive disease from three to six months) (Tables 2 and 3).
3. Survival of patients with adenocarcinoma almost doubled from four and a half to eight and a half months for patients on three drug combinations. The difference is not statistically significant (R. Simon; Tables 4 and 5).
4. Patients with epidermoid carcinoma and with large cell carcinoma showed no superiority for either program.

Disposition:

1. Small cell carcinoma: The study is replaced by a new protocol, incorporating the three drug combination (CTX + MTX + CCNU) as

Table 1. ST 53: Percent Distribution of 339 Patients by Cell Type

Type of Cancer	Regional Disease	Extensive Disease
Epidermoid	53	29
Small cell	23	27
Adenocarcinoma	15	25
Large cell	9	19

HH/OS

Table 2. ST 53: Survival of Patients with Regional Disease

Type of Cancer	Survival (median) Radiotherapy	Combination	p
Epidermoid	217	154	0.2
Small cell	211	224	0.57
Adenocarcinoma	260	128	0.33
Large cell	168	50	0.82
Total	218	152	0.82

HH/OS

Table 3. ST 53: Number of Patients with Limited Disease

Type of Cancer	Radiotherapy Entered	Alive	%	Combination Entered	Alive	%
Epidermoid	48	16	33	41	14	34
Small cell	20	7	45	19	6	31
Adenocarcinoma	12	6	50	12	5	42
Large cell	8	1	12	7	1	14
Total	88	32	36	80	26	32

HH/OS

Table 4. ST 53: Survival of Patients with Extensive Disease

Type of Cancer	Survival (median) Two Drug	Three Drug	p
Epidermoid	127	147	0.74
Small cell	154	226	0.22
Adenocarcinoma	137	261	0.20
Large cell	109	105	0.8
Total	121	182	

HH/OS

Table 5. ST 53: Number of Patients with Extensive Disease

Type of Cancer	Two Drug Combination Entered	Alive	%	Three Drug Combination Entered	Alive	%
Epidermoid	24	7	29	25	7	28
Small cell	21	5	24	25	5	20
Adenocarcinoma	19	7	37	23	12	52
Large cell	16	1	0.6	16	1	0.6
Total	81	20	25	89	25	27

HH/OS

standard therapy. All previously untreated patients receive adjuvant, intensive radiotherapy to the bulk of the tumor mass. Elective radiotherapy to the brain is given to a random group of patients in order to eradicate tumor in this "sanctuary," beyond the reach of chemotherapy with water soluble drugs.

A new, not mutually cross resistant, combination is piloted in patients with prior therapy, including vincristine + bleomycin + hexamethylmelamine. Twelve patients are thus far entered into the study by membership institutions of the Working Party.

2. Adenocarcinoma and large cell carcinoma: New protocols are designed for regional disease (three drug combination as adjuvant to radiotherapy) and for extensive disease (present three drug combination versus mutually non-cross resistant combination in a crossover design). The present study, ST 53, is continued for patients with extensive disease.

3. Epidermoid carcinoma: Immunosurveillance will be initiated for good risk patients after surgery for cure. Surgical adjuvant chemotherapy will be studied for patients with high risk of recurrent disease. CCNU will serve as study drug because it tops the list of single antineoplastic agents against bronchogenic epidermoid carcinoma. Patients with regional disease will be allocated at random to two dose schedules of radiotherapy. Patients with extensive disease will receive a new four drug regimen, developed at M.D. Anderson Hospital, including vincristine, bleomycin, cyclophosphamide and CCNU. All these protocols will represent joint projects of the Working Party for Therapy of Lung Cancer

1.22 Laminar Air Flow Project (VI 6X1 LA) Martin H. Cohen

For the period July 1, 1972 to March 19, 1973 there have been 360 patient days spent in the laminar air flow rooms. Recently we have started the study of intensive chemotherapy of bronchogenic carcinoma within the laminar air flow room and two patients have been admitted thus far. The protocol for the study of patients with bronchogenic carcinoma involves randomization to three arms: 1) intensive chemotherapy (drug doses 50% higher than conventional doses) plus prophylactic G.I. and nasopharyngeal antibiotics in the LAF rooms, 2) conventional chemotherapy plus G.I. prophylaxis on the open ward, and 3) chemotherapy with no antibiotics on the open ward. In addition, patients randomizing to the laminar air flow rooms are also randomized to receive either prophylactic tracheobronchial antibiotics delivered by nebulization or no prophylactic antibiotics. Preference is given to patients with bronchogenic small cell carcinoma, because of considerable responsiveness to antineoplastic chemotherapy.

2. Introduction of New Anticancer Agents into Clinical Use and Pharmacology of New Antitumor Agents

2.1 Diketopiperazine (NSC 129943; ICRF 159)

2.11 Phase I Trial (IV 4X1 LA) Patrick J. Creaven

Phase I trial on three schedules of administration is nearing completion. On a single dose, doses up to 10.5 g/m^2 were only mildly toxic (26 evaluable patients were treated). At 3.0 g/m^2 weekly, 6/7 achieved WBC < 5.0, 2/7 had WBC < 3.0. At 10 g/m^2 t.i.d. once weekly, 5/6 had WBC < 3.0. Two additional patients will be treated at this dose to conclude the study.

2.12 Clinical Pharmacology (IV 4X2 LA) Patrick J. Creaven and Larry M. Allen

In an initial clinical trial we have demonstrated a plateau in dose response of this drug above 3.3 g/m^2 given as a single dose. Investigations have been conducted to determine whether this could be due to impaired absorption. The absorption in 4 patients given 10.5 g/m^2 was 8.05% of the dose. In the same patients after 3.0 g/m^2, it was 22.7%. When given as a split dose t.i.d. in 4 patients, the absorption of 3 g/m^2 was 45.49%. Radioactivity not recovered in the urine appeared in the feces. The results clearly indicate that absorption is incomplete after large doses and indicate the need for a split dose schedule in further trials with this drug.

2.2 Epipodophyllotoxin Ethylidene Glucoside (NSC 141540)

2.21 Phase I Initial Clinical Trial (IV 4X1 LA) Patrick J. Creaven

Sixteen patients have been treated on the Phase I evaluation of weekly X 6 doses. Clinically inconsequential hematologic toxicity is noted at the present dose of 130 mg/m^2. It is anticipated that 130 or 170 mg/m^2 will be the recommended weekly dose. Five to 8 additional patients will be needed to complete the study.

2.22 Clinical Pharmacology (IV 4X2 LA) Patrick J. Creaven and Larry M. Allen

Interest is centered on penetration of drugs into the CNS because of reported activity against CNS leukemia. However, in examination of the C.S.F. in 4 patients at 4 to 48 hours after dosage with 70 or 100 mg/m^2 by i.v. infusion, no radioactivity was found, indicating essentially zero transfer of drugs across the blood brain barrier. Total recovery of radioactivity in the urine is only 45% of the total injected dose. The fate of the remaining 55% is still undetermined. Chromatography of the plasma and urine does not reveal the presence of detectable metabolites.

2.3 Isophosphamide (NSC 109724)

2.31 Preclinical Pharmacology (IIB 2B3 SA) Patrick J. Creaven and Larry M. Allen

Animal studies revealed that microsomal activation of isophosphamide had a K_m of five times the K_m of cyclosphosphamide activation, indicating a lower affinity for the microsomal enzyme in line with the clinically observed higher dose of isophosphamide required for the same effect.

2.32 Phase I Trial (IV 4X1 LA) Martin H. Cohen

This Phase I study has been completed and a manuscript is being prepared. The recommended dose is 5000 mg/m^2 i.v. q 3 w. The study showed that the major toxicity of isophosphamide was hemorrhagic cystitis. This could be prevented by bladder irrigation (with acetylcysteine). Hematologic toxicity was not dose limiting. Other toxicity included nausea and vomiting in all patients, central nervous system toxicity expressed as confusion and lethargy in 3 patients, mild BUN elevation in one patient, and mild alopecia in about 70% of the patients.

2.33 Clinical Pharmacology (IV 4X2 LA) Patrick J. Creaven and Larry M. Allen

Clinical pharmacology indicated that, at doses equitoxic to the marrow, the urinary alkylating activity after isophosphamide is significantly greater than that after cyclophosphamide, in keeping with the greater propensity of the former to cause bladder toxicity. Bladder toxicity was associated with higher concentration of alkylating activity (>800 m mole/HN_2 eg/ml) in the urine. Alkylating activity in the urine was <u>not</u> affected by acetylcysteine bladder washout.

2.4 Thalicarpine (NSC 68075)

2.41 Biochemical Pharmacology of Thalicarpine (I 1XX LA) Patrick J. Creaven and L.M. Allen

<u>Mode of Action</u>: Thalicarpine has been shown to inhibit DNA, RNA, and protein synthesis in cultured L1210 cells. A major block occurs at the level of thymidine triphosphate incorporation.

2.42 Phase I Trial (IV 4X1 LA) Patrick J. Creaven

Three patients have been treated at 200 mg/m^2 by i.v. infusion. No toxicity was noted. The next patient will receive 400 mg/m^2.

2.43 Interaction of Thalicarpine with Microsomal Enzymes (VI 6X2 SA)
Patrick J. Creaven and Larry M. Allen

Thalicarpine inhibits microsomal N-demethylation and aromatic hydroxylation. It does not affect either activity when animals are pretreated with it, but it prevents the induction of N-demethylation by phenobarbital and hydroxylation by methyl cholanthrene. It inhibits uridinediphosphoglucuronul transferase. It is clear that thalicarpine has a high potential for interaction with the metabolism of other drugs.

3. Improved Use of Chemotherapeutic Agents of Established Value

3.1 Enhancement of Nitrosoureas by Membrane Active Agents (IIA 2AX SA)
Martin H. Cohen

Previous work has shown that combining a variety of membrane active drugs, including vitamin A alcohol and several psychotropic drugs (the most effective being chlorpromazine), with caffeine markedly enhances the antitumor effect of nitrosoureas in murine leukemia L1210. Other drugs have been more recently tried in this system. Enhancement with hydroxyurea is not dose related and is similar whether hydroxyurea is given day 1, 3, or 5 before administration of BCNU or day 1 afterward. The lack of dose dependence makes it unlikely that the primary effect of treatment relates to inhibition of DNA synthesis. The manuscript of this study is in preparation.

3.2 Optimal Conditions for Combination Chemotherapy in Spin Culture Systems (IIA 2AX SA) Martin H. Cohen

Experiments using L1210 cells in culture and the Thayer-Himmelfarb spin filter apparatus are designed to demonstrate optimal timing for combination chemotherapy in culture. Nutrients to cultures are varied to develop a "fast" and "slow" growing cell line. Cell parameters, including DNA, RNA, and protein synthesis, are studied for each drug treatment and optimal time for the second drug in the combination determination from the changes in these results from the initial treatment. Presently we are working on the combination of hydroxyurea with a nitrosourea.

3.3 Phase I Study of Combination Chemotherapy with Chlorpromazine, CCNU, and Caffeine (IV 4X1 SA) Martin H. Cohen

The study was prompted by experiments in murine leukemia L1210 (submitted to the J. Natl. Cancer Inst. for publication) which showed that chlorpromazine and caffeine markedly enhance the antitumor effect of a nitrosourea. Thus, with BCNU alone there were no cures, while with the three-drug combination there were 70% cures.
Drug doses in man are chlorpromazine, 50 mg/m^2 i.m. 3 hours before CCNU; CCNU 130 mg/m^2 p.o.; and caffeine, 600 mg/m^2 s.c. 15 minutes after CCNU.
Five patients have been entered on study. There has been no increased hematologic toxicity associated with the combination com-

pared to that which would be expected with CCNU alone. There has been no toxicity from either chlorpromazine or caffeine administration.

3.4 Participation in Cooperative Studies (-- 8XX LA) (17 patients)

ALGB

#7122 - Comparison of Intermittent Cytosine Arabinoside plus Cyclophosphamide with Cytosine Arabinoside plus Daunorubicin in Induction and Maintenance of Untreated AML. (3 patients)

#7221 - Comparison of Daunorubicin with Daunorubicin plus Ara-C plus 6-TG and Ara-C plus 6-TG for Induction Therapy of AML and Comparison of CTX, HO-urea, and 6-MP or CTX, HO-urea, and 6-TG or CTX, HO-urea, 6-TG and 6-MP for Maintenance. (1 patient)

#7251 - A Study of Four-Drug Combination Chemotherapy (vinca alkaloids and alkylating agents) in Remission Induction and Maintenance Therapies of Stage III and IV Hodgkin's Disease. (1 patient

#7253 - Combination Chemotherapy of Stage III and IV Lymphosarcoma and Reticulum Cell Sarcoma - Induction and Maintenance. (1 patient)

#7254 - Treatment of Advanced Lymphosarcoma and Reticulum Cell Sarcoma with Adriamycin in Two Dose Schedules and with Prednisone Followed by Maintenance with Procarbazine Hydrochloride or Procarbazine Hydrochloride plus CCNU. (1 patient)

#7261 - Protocol for Primary Treatment of Multiple Myeloma. (1 patient)

#6953 - Comparison of BCNU and CCNU in the Treatment of Patients with Hodgkin's Disease. (1 patient)

#7121 - Comparison of Intermittent Ara-C + Cyclophosphamide with Ara-C + TG with Ara-C + CCNU Induction and Maintenance of Untreated AML. (3 patients)

ECOG

EST 0270 - Dactinomycin (NSC 3053) and Mithromycin (NSC 24559) in Patients with Hypercalcemia. (4 patients)

EST 4472 - Intermittent BCNU, CTX, and Prednisone vs. Intermittent Melphalan and Prednisone in Myeloma. (1 patient)

4.1 Statistics

Disease Categories of Patients Treated

Bronchogenic carcinoma	109
Gastrointestinal carcinoma	10
Acute leukemia	6
Genitourinary carcinoma	5
Hodgkin's disease	5
Lymphoma	5
Lymphosarcoma	4
Head and neck carcinoma	3
Myeloma	2
CNS	1
Melanoma	1
Pancytopenia	1
	152

Patient Census Data

On March 30, 1973: In-Patients, 24; Out-Patients, 75 = 99, total

From April 1, 1972 to March 31, 1973:

Admissions	289
Discharges	278
Total In-Patients	130
Total Out-Patients	145
Average Daily Patient Census	23.5
Out-Patient Visits	1682
Consults	263
Deaths	93
No. of Lab Tests (General Lab, WVAH)	34,075

4.2 Total Patient Care

Intensive chemotherapy is most effective on the basis of optimal total care. The members of the health team involved in total care interchange intensively. The following reports are submitted for each sector, with the exception of activities which are not supported by NCI transfer funds (radiotherapy, surgery, general laboratories, transportation, and chaplaincy service).

4.21 Nursing Activities

During the past year emphasis on sputum cytology has required nursing staff personnel to perform pulmonary therapy in selected patients for pretreatment deep-cough specimens and post-treatment sequential specimens. Absorption studies on selected

patients are carried out by nursing staff. Monthly meetings discussing patient needs are attended by nursing staff, social worker, and chaplain. Patients and/or family members are included when appropriate.

4.22 Social Work Service Nan Miller

The social worker, NCI-VA Medical Oncology Branch, provided direct and indirect social services to patients admitted to the unit as well as to out-patients who are being followed by the Oncology clinics. It is necessary that the social worker make a skilled appraisal of the social and emotional complications which are affecting the patient. She was avilable to assist the patient and his family in resolving personal and environmental problems relating to his illness, his continuing disablement, and/or his discharge from the hospital, using hospital, community, and other resources.

4.23 Dietetic Service Sadie Arkwright

The dietitian and two dietary technicians provide dietetic service to the patients on the Oncology unit. All patients are visited daily; individual attention is given to each patient concerning his dietary needs. The dietitian collects detailed diet histories on all patients with bronchogenic carcinoma, using specially designed forms to provide information on the relationship of nutritional status and weight change to prognosis. The study is designed to lead into more extensive study on mechanisms responsible for weight-loss in patients with malignant disease. As etiologic factors of cachexia are identified it is hoped the specific treatment to reverse these changes can be used.

In selected patients we are also exploring hyperalimentation, whether by the intravenous or oral route. For the latter, the use of solutions of essential amino acids, medium chain triglycerides, and non-lactose containing carbohydrates are being investigated.

The aseptic techniques instituted for serving patients in the Laminar Air Flow units have worked well. The "cooked food" diet served on a sterile tray is still being used. Informal lectures and tours have been conducted throughout the year. Twelve dietetic interns from Andrews Air Force Base, ten dietitians from other hospitals, and three management analyst trainees visited to observe the dietary management of the reverse isolation units. The dietitian conducted extensive training sessions in aseptic techniques to employees assigned to the unit and to other dietetic employees who may perform relief duties in the future. The Oncology technicians were also trained in calculating food intake.

The dietitian worked closely with the Assistant Chief, Dietary Service, in investigating sources of supply of food items, not usually purchase by the hospital, for the Laminar Air Flow units.

4.24 Educational Activities

Optimal total care is greatly facilitated by on-site as well as formal training activities, including, among others the following:

Medical Staff:

Medical staff members attended and participated in regularly scheduled seminars, rounds, conferences, and Tumor Board at the VA Hospital and the National Cancer Institute. Staff physicians contributed ten months of attending coverage on the VA Hospital medical wards.

Three trainees have completed two years; two spent one month each at the Walter Reed Hospital Hematology-Oncology Department, the remaining time in the Medical Oncology Branch. There were six first-year trainees participating in the Medical Oncology Branch program, on a rotating basis, including one from the Cancer Therapy Evaluation Branch. Two George Washington University Medical students spent two months each on the Oncology ward, supervised by Dr. Martin Cohen. Two sophomore medical students from George Washington University are assigned to Dr. M. Matthews for training in organ and tumor pathology. Two resident electives from Georgetown University and two from George Washington University for 1, 1, 1, and 4 months, respectively, participated in the training program.

Biweekly conferences in pathology (Dr. Matthews) and pharmacology (Dr. Creaven) were held for all interested medical staff. Weekly Oncology conferences and rounds were held, featuring scheduled outside and on-staff speakers on topics related to the Oncology Branch program. These conferences were attended by an average of twenty-five participants.

Dr. Matthews participated in a one-week course on "Histochemical Diagnostic Techniques," conducted by Dr. Frank Johnson, AFIP, Washington, D.C., January, 1973. Dr. Matthews participated in the "Medical Writing Seminar," conducted by Dr. Guy Whitehead at the International Academy of Pathology, Washington, D.C., March, 1973. Dr. Suryabala Kanhouwa attended the course, "Clinical Cytopathology for Pathologists," conducted by Dr. John Frost, Johns Hopkins University.

Dr. Larry Allen, Research Pharmacologist, attended courses on: Cellular Aspects of Immunology; Mass Spectrometry; Accessing Pre-Packaged Statistical Routines; CPS Introduction; and MLAB, Modelling, Graphics, and Displays on PDP10.

Nursing Staff:

Nursing personnel attended and participated in the VA Hospital Nursing Service training programs. Nurses were encouraged to attend the weekly Oncology conferences and rounds and daily working rounds. Weekly patient-oriented discussions, emphasizing pathophysiology of cancer and clinical management, were led by medical or nursing staff members. Dr. Creaven held a series of lectures on Phase I agents for the nursing staff.

Ms. West, NA, attended a one-day conference on "Cancer in Children." Ms. M. Boyer, Head Nurse, attended one day of the First International Workshop for Therapy of Lung Cancer. Junior level nursing students from Georgetown University and graduate students from Catholic University are assigned to the Medical Oncology Branch Nursing Staff for training and experience.

Oncology Supporting Staff:

Ms. S. Arkwright, dietitian, completed four courses at Howard University: Food Economics, Problems of the Administrative Dietitian, The Administrative Dietitian, and Special Problems in Home Economics. She attended the Annual Meeting of the American Dietetic Association; the National Dairy Council Workshop, and The D.C. Dietetic Workshop on Medical, Nutrition and Social Aspects of the Cancer Patient.
Ms. E. Mackie, Administrative Assistant, attended the Administrative Officer Seminar.
Ms. S. Anglade, Secretary, attended the Executive Secretary Seminar.

5. Honors and Awards

Dr. Allen

1. "Induction of Isophosphamide Metabolism by Phenobarbital," presented at the 5th International Congress of Pharmacology, San Francisco, California, July, 1972. Proc. 5th International Congress of Pharmacology 1:4, 1972.
2. Allen, L.M., and Creaven, P.J. The interaction between proteins and antineoplastic alkylating agents. Proc. 164th ACS National Meeting Biol. p. 145, 1972.
3. "Studies on a New Cytoxan Analog, Isophosphamide," presented at the American Federation for Cancer Research, D.C. Chapter, Georgetown University, December, 1972.
4. "Interaction Between DNA and a New Oxazaphosphorine Nitrogen Mustard," presented at the American Chemical Society Mid Atlantic Meeting, Washinton, D.C., January, 1973. Proc. 164th ACS National Meeting Biol. 148, 1972.
5. Allen, L.M., Owens, C., and Creaven, P.J. Determination of plasma isophosphamide by gas chromatography. Proc. ACS Middle Atlantic Regional Meeting p. 60, 1973.
6. "Thalicarpine Inhibition of DNA, RNA, and Protein Synthesis in Cultured Mouse Leukemia Cells (L1210)," presented at the AACR Meeting, Atlantic City, April, 1973. Proc. Amer. Assoc. Cancer Res. 14:36, 1973.
7. Membership in: The American Chemical Society

1. "Eastern Cooperative Oncology Group : Recent Studies in Lung Cancer," presented at the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
2. "Use of Fiberoptic Bronchoscopy as a Method for Following Response," presented at the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
3. "Combination Chemotherapy and Radiotherapy in Lung Cancer," presented at the American Society of Therapeutic Radiologists, Phoenix, Arizona, November, 1972.
4. "Use of Isophosphamide in Adult and Childhood Solid Tumors," presented at the Acute Leukemia Group B and American Society of Hematology, Hollywood, Florida, December, 1972.
5. "Plans for Chemotherapy in a Protected Environment," presented at the 4th Conjoint Meeting, Annapolis, Maryland, January, 1973.
6. Cohen, M.H. Initial clinical trials with isophosphamide. Proc. Amer. Assoc. Cancer Res. 14:253, 1973.
7. Appointed Executive Officer, Eastern Cooperative Oncology Group.
8. Memberships in : American Society of Hematology
   Society of Experimental Hematology
   American Association for Cancer Research
   American Society of Clinical Oncology
   Acute Leukemia Group B
   Education Committee, NCI
   Contract Review Committee, NCI

Dr. Creaven

1. "Renal Clearance of Camptothecin Sodium in Man: Effect of Urine Volume," presented at the 5th International Congress on Pharmacology, San Francisco, California, July, 1972. Proc. 5th Internat. Cong. Pharmacol. 1:481, 1972.
2. "The Interaction Between Proteins and Antineoplastic Alkylating Agents," presented at the Annual Meeting of the American Chemical Society, New York, August, 1972. Proc. 164th ACS Natl. Meeting Biol. 145, 1972.
3. "Urinary Alkylative Activity after I.V. Isophosphamide in Man," presented at the 9th Pan-American Congress of Pharmacy and Biochemistry, Panama, November, 1972. Proc. 9th Pan American Cong. Pharm. Biochem. 1:41, 1972.
4. "The Interaction of Thalicarpine with Rat Liver Microsomes," presented at the American Chemical Society Mid Atlantic Meeting, Washington, D.C., January, 1973. Proc. ACS Middle Atlantic Regional Meeting I:60, 1973.
5. "Isophosphamide Pharmacology," presented at the Phase I-Phase II Liaison Meeting, NCI, Bethesda, February, 1973.
6. "ICRF 159, Phase I Study," presented at the Phase I-Phase II Liaison Meeting, NCI, Bethesda, February, 1973.
7. "Clinical Pharmacology of Isophosphamide Correlation with Preclinical Studies," presented at the Department of Pharmacology Seminar, George Washington University, Washington, D.C., March, 1973.
8. Creaven, P.J., and Taylor, S.G., III. Phase I evaluation of single and weekly doses of (±)1,2-bis-(3,5 dioxopiperazin-1-yl-propane. Proc. Amer. Assoc. Cancer Res. 14:79, 1973.

9. Elected a Fellow of the Royal Society of Health, Great Britain
Secretary-Treasurer, D.C. Chapter, American Federation for Clinical Research
Membership in : Biochemical Society
New York Academy of Sciences
American Association for the Advancement of Science
Academy of Pharmaceutical Sciences
American Federation for Clinical Research
Acute Leukemia Group B
Eastern Cooperative Oncology Group

Dr. Hansen

1. "A Randomized Study of Two- versus Three-Drug Combination Chemotherapy in Bronchogenic Carcinoma : Preliminary Data," presented at the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
2. "Randomized Study of Combination Treatment of Small Cell Carcinoma," presented at the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
3. "Evaluation of Hepatic Involvement in Lung Cancer," presented at the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
4. "Role of CCNU in Combination Chemotherapy," presented at the 4th Conjoint Meeting, Annapolis, Maryland, January, 1973.
5. "Advances in Staging," presented at the 4th Conjoint Meeting, Annapolis, Maryland, January, 1973.
6. Muggia, F.M., Rosen, S.W., Wintraub, B.D., and Hansen, H.H. Ectopic placental hormones in nontrophoblastic tumors : Serial measurements following chemotherapy. Proc. Amer. Assoc. Cancer Res. 14:78, 1973.
7. Appointed Executive Officer for the Working Party for Therapy of Lung Cancer.
8. Site Visits: Wayne State University, Detroit, Michigan; M.D. Anderson Hospital and Tumor Institute, Houston, Texas.
9. Memberships in: American Association of Hematology
American Association of Cancer Research
American Association of Clinical Oncology
Acute Leukemia Group B
Eastern Cooperative Oncology Group
American Federation for Clinical Research

Dr. Kanhouwa

1. "Radiation Pathology," presented at the AFIP, Washington, D.C., September 26, 1972.
2. Participated in the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
3. "Frequency of Residual and Metastatic Disease in Curative Lung Resections," presented at George Washington University, November, 1972.

4. "Incidence of Metastatic Disease in Patients Dying 30 Days Post-Operative for Curative Lung Resection," presented at the 4th Conjoint Meeting, Status of Clinical Studies, Division of Cancer Treatment, Annapolis, Maryland, January, 1973.

5. Membership in: Washington Society of Pathologists
American Medical Association
Tropical Diseases Society
American Society of Clinical Pathologists
American Board of Pathology

Dr. Matthews

1. "Serum-lipose Producing Acinar Cell Carcinoma of the Pancreas: Clinical-Pathologic Correlation and Electron Microscopic Study," presented at the VA Hospital, Martinsburg, West Virginia, September, 1972.
2. Coordinator for the Pathology Sessions at the First International Workshop on Lung Cancer at Airlie, Virginia, October 16-20, 1972. Drs. Matthews, Kanhouwa, and John Pickren, Roswell Park Memorial Institute, presented findings on frequency of residual or metastatic disease in patients who had undergone curative resections for lung cancer and died within one month post-operatively. Dr. Matthews presented:
"Evaluation of Accuracy of Staging Procedures in Patients with Lung Cancer," "Keynote Address" for the Pathology Session, "Frequency of Residual and Metastatic Tumor in Patients Undergoing Curative Surgical Resection for Lung Cancer."
3. "Working Party for Therapy of Lung Cancer: A Multidisciplinary Approach," presented at the Pathology Research Seminar, George Washington University, November, 1972.
4. "Consistancy in Morphologic Diagnosis in Lung Tumors," presented at the 4th Conjoint Meeting, Annapolis, Maryland, January, 1973.
5. Becker, K., Matthews, M.J., Higgins, G., and Mohamadi, M. Prospective study of the gynecomastia associated with hyperthyroidism. Fed. Proc. Exper. Path. 32 216, 1973.
6. Chairman, Registrations, for the meeting of the American Association of Pathologists and Bacteriologists and International Academy of Pathology, Washington, D.C., February-March, 1973.
7. Appointed to the Executive Committee, Washington Society of Pathologists, 1972-1973.
8. Appointed Chairman, Arrangements Committee, Combined Washington and Maryland Societies of Pathology, Annual Meeting, Annapolis, Md., 1973.
9. Memberships in: American Society of Cytology
American Medical Association
International Academy of Pathologists
American Association for Advancement of Science
American Medical Women's Association
10. As chairman of the Pathology Committee of the Working Party for Therapy of Lung Cancer, Dr. Matthews held three committee meetings during the year, presenting reports to the general meetings. Major effort of the committee was in the area of classification: working out pathology and autopsy forms, and classification of tumor types. Extensive discussions concerning reviewer agreement took place.

1. "Advances in Staging," presented at the 4th Conjoint Meeting, Annapolis, Md., January, 1973.
2. "Progress Report on VP16," presented at the Phase I-Phase II Liaison Meeting, NCI, Bethesda, Md., February, 1973.
3. "High Incidence of Development of Brain Metastases During Treatment of Patients with Small Cell Carcinoma of the Lung," presented at the American Society of Clinical Oncology Meeting, Atlantic City, April, 1973. Proc. Amer. Soc. Clin. Oncol. No. 48, 1973.

Dr. Primack

1. "The Management of Hepatocellular Carcinoma," presented in Kuala, Lumpur, Malaysia, September, 1972.
2. Participated in the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
3. "Staging and Prognoses of Hepatocellular Carcinoma in Uganda," presented at the American Federation for Clinical Research Regional Meeting, Washington, D.C., February, 1973.
4. Guest editor, Cancer Chemotherapy Reports Symposium on Therapy of Lung Cancer, March, 1973.
5. "Staging of Hepatocellular Carcinoma," presented at the American Association for Cancer Research Meeting, Atlantic City, April, 1973. Proc. Amer. Assoc. Cancer Res. 14:11, 1973.
6. Membership in: American Society for Clinical Oncology
   American Association for Cancer Research
   American Federation for Cancer Research

Dr. Selawry

1. "Progress in Chemotherapy," presented at the Washington Hematology Review Course, Washington, D.C., September, 1972.
2. "Principles of Chemotherapy," presented at the Walter Reed General Hospital, Washington, D.C., October, 1972.
3. "Bronchogenic Carcinoma: An Overview of Therapy Plans," presented at the Contractors Meeting, Hunt Valley, Md., October, 1972.
4. "General Methodological Considerations in the Planning of Therapeutic Trials for Bronchogenic Carcinoma," "Single Agent Chemotherapy as Related to Cell Type," "Keynote Address," presented at the First International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
5. "Implications of Cell Types for Differential Therapy of Carcinoma of the Lung," presented at the American College of Chest Physicians Meeting, Baltimore, Md., November, 1972.
6. "Current Plans For Studies," 4th Conjoint Meeting, Annapolis, Md., January, 1973.
7. "Lung Cancer," presented at the Goerge Washington University Medical Center, Washington, D.C., February, 1973.

8. "Lung Cancer," presented at the Walter Reed General Hospital, Washington, D.C., February, 1973.
9. "Lung Cancer Therapy at the Medical Oncology Branch, NCI-VA, and at The Working Party for Therapy of Lung Cancer," presented at the Eastern Oncology Group Meeting, Miami, Florida, March, 1973.
10. "Chemotherapy in Treatment of Lung Cancer," presented at the Prince Georges County Hospital, May 17, 1973.
11. "New and Promising Agents and Combination for Treatment of Lung Cancer," a course given in Medical Oncology and Chemotherapy at the University of Southern California, Los Angeles, California, June, 1973.
12. "Possibilities for More Effective Treatment of Bronchogenic Carcinoma," presented at the celebration of the 20th anniversary of the Cancer Research Institute of the University of Vienna, Vienna, Austria.
13. Dr. Selawry was invited to participate in the initial negotiations for cooperation in the field of cancer therapy between the USA and the USSR in Moscow from June 25 to June 30, 1972. While there, he presented "Mono- and Multi-disciplinary Therapy of Lung Cancer by Cell Type" at the Institute for Experimental and Clinical Oncology of the Soviet Academy of Medical Sciences.
    Along with two other members of the Division of Cancer Treatment, Dr. Selawry was invited to participate in a site visit to four leading clinical cancer research centers in the Soviet Union for detailed exploration of mutual cooperation in cancer therapy, November 26 to December 12, 1972. Lectures given:
    "The Initial Clinical Trial of New Antineoplastic Agents," presented at the Institute for Experimental and Clinical Oncology of the Soviet Academy of Medical Sciences, Moscow, December 1, 1972.
    "Current Approaches to Multidisciplinary Therapy of Lung Cancer," presented at the Institute of Problems of Oncology of the Ukrainian Academy of Medical Sciences, Kiev, December 6, 1972.
    "The Role of Chemotherapy in the Treatment of Lung Cancer," presented at the Petrov Oncological Institute of the Soviet Ministry of Health, Leningrad, December 10, 1972.
    Dr. Selawry was invited to participate in the All-Russian Scientific Medical Society of Oncologists Conference on Malignant Melanoma, Herzen Oncological Institute of the Russian Federal Soviet Republic, Moscow, December 11, 1972.
14. Chairman, Ad Hoc Committee for Evaluation of Proposals for Therapy of Lung Cancer
    Site Visits: Ohio State University, M.D. Anderson Hospital and Tumor Institute, University of Tennessee.
15. Chairman, Working Party for Therapy of Lung Cancer
    Chairman, International Workshop on Therapy of Lung Cancer
16. Site Visits to: University of Liverpool, England; University of Vienna, Austria; and the University of Cape Town, South Africa, May 23 - June 10, 1973.

17. Memberships: American Association for Cancer Research
American Federation for Clinical Research
New York Academy of Science
American Society of Clinical Oncology
American Society of Hematology
American Federation of Clinical Oncologic Societies
Acute Leukemia Group B
Eastern Cooperative Oncology Group
NCI Operations Committee, NCI
Decision Network Committee, NCI
Experimental Design Committee, NCI
Specialty Fellowship Board, NCI
Veterans Administration Merit Review Board for Oncology

Dr. Straus

1. "Lung Cancer" presented at Grand Rounds, Barnes Hospital, St. Louis, Mo., July, 1972.
2. "The Application of Cell Kinetics to the Therapy of Lung Cancer" presented at Grand Rounds, City Hospital of St. Louis, St. Louis, Mo., August, 1972.
3. "The Treatment of Oat Cell Carcinoma" presented at the Pulmonary Division. Jewish Hospital of St. Louis, St. Louis, Mo., September, 1972.
4. "Introduction to Cancer Chemotherapy" presented to second year medical students, Washington University School of Medicine, St. Louis, Mo., July, 1972.
5. "Tumor Cell Kinetics" presented at cell biology course, Barnes Hospital, St. Louis, Mo., August, 1972.
6. "Diagnosis and Treatment of Lung Cancer" presented at Pulmonary Division, Barnes Hospital, St. Louis, Mo., September, 1972.
7. "Experimental Models for the Study of Cell Kinetics" presented at Grand Rounds, Washington V.A. Hospital, Washington, D.C., March, 1973.
8. "Cell Kinetics and Its Application for Chemotherapy and Immunotherapy Designs in Patients" presented at Grand Rounds, Washington V.A. Hospital, Washington, D.C., March, 1973.
9. Participated in the International Workshop for Therapy of Lung Cancer, Airlie, Virginia, October, 1972.
10. Executive Officer, NCI Working Party for Therapy of Lung Cancer.
11. Memberships: Phi Alpha Theta - National History Honor Society
Pi Gammu Mu - National Social Science Honor Society
American Association for the History of Medicine
American Association for Cancer Research
Associate, American College of Physicians
Member, Acute Leukemia Group B
American Federation for Clinical Research

6. Publications (-- 8XX)

1. Allen, L.M. and Creaven, P.J. Activation of the antineoplastic drug

isophosphamide by rat liver microsomes. J. Pharm. Pharmacol. 24: 585, 1972.

2. Allen, L.M. and Creaven, P.J. A gas chromatographic method for the determination of plasma isophosphamide (NSC 109724). Cancer Chemother. Rep. (In press).
3. Allen, L.M. and Creaven, P.J. Effect of microsomal activation on interaction between isophosphamide and DNA. J. Pharmacol. Sci. 61:2009-2011, 1972.
4. Allen, L.M. and Creaven, P.J. In vitro liver activation of isophosphamide (NSC 109724): A new oxazaphosphorine. Cancer Chemother. Rep. 56:603-610, 1972.
5. Allen, L.M. and Creaven, P.J. On the interaction of mechlorethamine and isophosphamide with bovine serum albumin and rat liver microsomes. J. Pharm. Sci. (In press).
6. Broder, L.E. and Hansen, H.H. 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC 79037): A comparison of drug administration at four-week and six-week intervals. Eur. J. Cancer 9:147-152, 1973.
7. Creaven, P.J., Allen, L.M. and Muggia, F.M. Plasma levels during a 5-day course. Cancer Chemother. Rep. 56:573-578, 1972.
8. Creaven, P.J., Burns, W.A. and Selawry, O.S. Widespread metastases and renal failure from carcinoma of the soft palate: Report of a case. J. Urol. 108:223-226, 1972.
9. Freis, E.O., Roger, D., Pillsburg, H., Matthews, M.J. Alteration of the course of hypertension in the spontaneous hypertensive rat. Cir. Res. 31:1-7, 1972.
10. Gallico, G.E., Vogel, C.L., Kiryaburre, J.W.M., Primack, A., Dhru, D. and Kamya, G.W.S. Recent advances in malignant melanoma research in Uganda. Uganda Med. J. (In press).
11. Hansen, H.H., Kanhouwa, S. and Margolis, R. Liver metastasis in bronchogenic carcinoma. Cancer Chemother. Rep. (In press).
12. Hansen, H.H. and Muggia, F.M. Staging of inoperable patients with bronchogenic carcinoma with special reference to bone marrow and peritoneoscopy. Cancer 30:1394-1401, 1972.
13. Hansen, H.H., Muggia, F.M., Andrews, R.J. and Selawry, O.S. Intensive combined chemotherapy and radiotherapy in patients with nonresectable bronchogenic carcinoma. Cancer 30:315-324, 1972.
14. Hansen, H.H., Muggia, F.M. and Napoli, L. The incidence of bone metastases in lung cancer with special reference to the development of osteoblastic metastases. Radiol. (In press).
15. Matthews, M.J. Keynotes of pathology of lung cancer. Cancer Chemother. Rep. (In press).
16. Matthews, M.J. Morphologic classification of bronchogenic carcinoma: Summary and statement of pathology panel. Cancer Chemother. Rep. (In press).
17. Matthews, M.J., Kanhouwa, S., Pickeren, J., Robinette, D. Frequency of residual and metastatic tumor in patients undergoing curative surgical resection for lung carcinoma. Cancer Chemother. Rep. (In press).
18. Muggia, F.M., Creaven, P.J., Hansen, H.H., Cohen, M.H. and Selawry,O.S. Phase I clinical trial of weekly and daily schedules of camptothecin

sodium (NSC 100800): Correlation with preclinical studies. Cancer Chemother. Rep. 56:515-521, 1972.

19. Muggia, F.M. and Hansen, H.H. Osteoblastic metastases in small-cell (oat cell) carcinoma of the lung. Cancer 30:801-805, 1972.
20. Primack, A., Dhru, D., Kiryabuure, J.W.M., and Vogel, C.L. Clinical trials of combination chemotherapy with BCNU and vincristine in the treatment of malignant melanoma in Uganda. Cancer 31:337-341, 1973.
21. Primack, A., Vogel, C.L. and Barker, L.F. Immunological studies in Ugandan patients with hepatocellular carcinoma. Brit. Med. J. 1:16-19, 1973.
22. Ohnuma, T., Selawry, O.S., Holland, J.F., DeVita, V.T., Shedd, D.P., Hansen, H.H. and Muggia, F.M. Clinical study with bleomycin: Tolerance to twice weekly dosage. Cancer 30:914-922, 1972.
23. Sauerbrunn, B.J., Hansen, H.H. and Napoli, L. Strontium bone scans in carcinoma of the lung. A comparative prospective study. J. Nucl. Med. 13:465-466, 1972.
24. Selawry, O.S. Initial therapeutic trial of new drugs in lung cancer. Cancer Chemother. Rep. (In press).
25. Selawry, O.S. Introduction to the first International Workshop on Lung Cancer. Cancer Chemother. Rep. (In press)
26. Selawry, O.S. Lung cancer. Cancer Chemother. Rep. (In press).
27. Selawry, O.S. Monochemotherapy of bronchogenic carcinoma with special reference to cell type. Cancer Chemother. Rep. (In press).
28. Selawry, O.S. and Hansen, H.H. Lung Cancer. In Cancer Medicine. Philadelphia, Lea and Febiger (In press).
29. Selawry, O.S. and Primack, A. (Eds.) Cancer Chemother. Rep. Vol. 4, Part 3, 1973 (In press).
30. Straus , M.J. Increased lifespan in oat cell carcinoma patients with a new system of altering drug doses. Proc. Amer. Assoc. for Cancer Res. (In press).
31. Straus , M.J. and Choi, S.C. Increased height associated with hypertension. J.A.M.A. 223:440-441, 1973.
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SUMMARY

OFFICE OF THE CHIEF

BALTIMORE CANCER RESEARCH CENTER

The combined clinical and laboratory investigative programs of the BCRC have had an extremely productive year with the publication of 52 papers in a broad spectrum of journals, approximately 16,000 patient treatment days, and the development of a number of new programs.

Phase I studies have been completed on Streptozotocin and IV Methyl CCNU, including human pharmacodynamics of the latter drug. Significant advances in the treatment of metastatic sarcoma with Adriamycin have been demonstrated. These studies have been complemented by pharmacokinetic and enzymologic evaluation. The metabolism of Adriamycin has been found to be different from that of Daunorubicin and is highly dependent upon hepatic function. Phase II studies in the treatment of malignant brain tumor have been completed evaluating oral Methyl CCNU, as well as intravenous Epipodophyllotoxin, and studies in ANLL utilizing Azacytidine and Guanazole are being conducted.

Controlled Phase III studies utilizing Daunorubicin, Ara-C, Thioguanine and Pyrimethamine in adult ANLL have shown significant value. The combination utilizes Phrimethamine specifically for the control of occult CNS leukemia. Combined modalities treatment (surgery, radiotherapy and chemotherapy) in Hodgkin's Disease and Non-Hodgkin's lymphoma have shown therapeutic advances and developed valuable data related to these diseases. Programs in the treatment of testicular carcinoma, malignant carcinoid and pancreatic tumors have been developed and are underway.

Phase III studies of protected environment (Laminar Air Flow Units), complemented by detailed microbiologic surveillance of both environment and patients, has led to an understanding of the microbiological impact upon the patient, and indicated ways of altering environmental balance in favor of the patient. Strict protocol control of antibiotic utilization has resulted in a significant decrease in mortality from pseudomonas infection and control of super infection. Viral diagnostic sureillance has noted a 10% incidence of herpes simplex virus in our patient population.

The evaluation of biologic markers of malignant disease have included muramidase, alpha fetoprotein, CEA, and plasma and urinary polyamine determinations. CEA and muramidase are evaluated in our own laboratories and current studies attempt to

relate polyamine concentrations to tumor burden utilizing the greatly increased sensitivity provided by the amino acid analyzer. The physiologic role of polyamines in normal and neoplastic tissue have been further elucidated.

Coordinated and integrated programs of infectious disease control, cell component therapy (platelets and white blood cells) and cancer nursing service provide the support necessary for these critically ill patients. The Patient Care Pharmacy provides detailed drug control through the use of unit dose service in the preparation of parenteral drugs for all patients. The correlation of massive amounts of clinical, pharmaceutical, microbiologic and therapeutic data has been achieved through the development of computer resources for census coordination, microbiologic reporting, and automated monitoring of drug therapy.

Combined laboratory of pharmacology and medical oncology service studies have involved the pharmacologic evaluation of Adriamycin, Daunomycin, oral Methyl CCNU, and IV Methyl CCNU. Detailed studies of the anthracycline antibiotics have identified a number of metabolites and the pharmacologic effects of glycosidase responsible for the metabolism of these substances. Studies of cellular control mechanisms and evaluation of sterol markers are being futher developed. The relationship of polyamines to RNA metabolism, the sequencing of RNA isolated from murine leukemia virus, and improved sensitivity in the instrumentation for polyamine determination are all being undertaken. Specific bacterial and mammalian nucleases and viral RNA isolated from AKR murine leukemia are being investigated. Large macromolecules have been partially identified. Immunologic research has been directed toward the detection of host response to specific antigens and the marked amplification of this responsiveness using poly AU. The use of such amplification in immunotherapy and immunodiagnosis is being evaluated. Studies of immune complex disease have indicated that some subclinical immune complex deposition occurs in a significant number of patients, most specifically in those harboring leukemia.

The Baltimore Cancer Research Center's integrated programs of clinical and laboratory investigation continue to provide a maximal therapeutic and investigative effort in the control of cancer. Participation within the Baltimore medical and scientific academic community have provided an excellent source of patient referral and scientific enrichment. An increase in the program with a gradual shift toward less well-controlled solid tumors is anticipated over the course of the next year.

Specific investigations into gynecologic and urologic cancers, as well as head and neck tumors will be undertaken, and additional Phase I and II studies will be added to the ongoing program. Although indications of the closure of the USPHS Hospital adds some uncertainty to geographic location, it is anticipated that minimal loss of scientific productivity will be encountered.

SUMMARY REPORT

Clinical Branch, Baltimore Cancer Research Center, DCT, NCI

July 1, 1972 through June 30, 1973

This year has seen significant progress on many fronts. Phase I studies with streptozotocin have been completed. Phase II studies have demonstrated progress in the control of metastatic sarcoma utilizing adriamycin according to dosages and schedules determined by pharmacokinetic studies. Phase II trials with new agents such as azacytidine and guanazole in acute non-lymphocytic leukemia have been initiated. Phase II studies of streptozotocin in malignant carcinoid and islet cell tumor have begun to bear fruit. Phase III studies in adult acute non-lymphocutic leukemia, adult acute lymphocytic leukemia, disseminated Hodgkins Disease and non-Hodgkin's lymphoma have produced valuable data. In fact, our current Phase III study in adult acute non-lymphocytic leukemia is producing some of the best therapeutic data in that disease available anywhere to date. Combined modality therapy studies in Hodgkin's disease utilizing surgery, radiation therapy, and chemotherapy show promise of significantly advancing the results of therapy in previously untreated patients with Hodgkin's Disease confined to lymph nodes.

Participation in oncology programs at the two medical schools in Baltimore and in other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community the impact of the DCT program.

Phase II studies of IV Methyl CCNU in brain tumors have been initiated. In addition, Phase II studies with epipodophyllotoxin (PTG) and oral Methyl CCNU have been successfully concluded. Thus, activity of several kinds of agents in malignant brain tumors have been identified.

Protected environment (Laminar Air Flow) studies have been enhanced by detailed microbiologic surveillance and diagnostic support. This effort has led to an understanding of the microbiologic environmental impact upon the patient and has indicated ways of altering the environmental balance in favor of the patient. Specific studies have examined pseudomonas infection, colonization of the environment and improved culture taking and retrieval systems. Viral diagnostic surveillance is being studied in detail for its efficacy with respect to the management of the immune suppressed host. Biologic markers of malignant disease such as muramidase, alpha fetoprotein, carcinoembryonic antigen, and plasma and urinary polyamines have been studied in detail and the data correlated with the type and extent of malignant disease.

This mass of data has been prepared for analysis and analyzed by a pilot computer-organized problem-oriented medical system. Doctors, nurses, pharmacists, and other related personnel that take part in the patient's care or his study contribute their input to the record through this computerized system.

Plans for the subsequent fiscal year include Phase III studies in metastatic sarcoma and a multitude of other Phase I and II studies in a variety of solid tumors. In addition, the role of immunotherapy in the maintenance of remission in leukemia patients will be explored.

Serial No.: NCI-6027
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Microbiological Services for Clinical and Research Studies for Cancer Patients at BCRC and at NIH, Clinical Center by Contracted Laboratory Facilities

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Robert C. Good, Ph.D., Robert W. Chambers, M.D., Norman R. Friedman, B.S., Dolores M. Kenton, B.S., Geetha S. Sebastian, M.T., I. J. Abramson, Ph.D., Arthur S. Levine, M.D., Cephus Patch, Ph.D., Janet Hauser, B.S.

Cooperating Units: Medical Service, NCI, BCRC. Leukemia Service, National Cancer Institute, N.I.H., Bethesda, Maryland, Hazleton Laboratories, Vienna, Virginia and Viral Diagnostics Laboratory, Georgetown University Hospital, Georgetown University, Washington, D.C.

Man Years (July 1, 1972 - June 30, 1973)

Total: 4 1/2
Professional: 4 1/2
Other: 0

Project Description:

Objectives:

1. To supply viral and bacterial diagnostic services of a high quality for the study of actual and potential infection of cancer patients and of a versatility necessary to support cancer chemotherapy research programs.

2. To carry out studies of the environment as well as surveillance cultures of the patient flora in an effort to provide microbiologically controlled Laminar Air Flow Rooms and information on the relationship of organisms found in the environment to those harbored by the patient.

3. To investigate the behavior of viral agents in immunosuppressed patients whose bacterial flora has been suppressed by oral, non-absorbable antibiotics.

Methods Employed:

Samples for bacterial, viral and environmental studies are collected in transport media and/or on primary culture media. They are transported daily by chauffeur driven government vehicle to the contracted bacteriological or viral diagnostic laboratories. Presumptive results and all other new data are sent twice daily by teletype or shared computer facilities. Final reports are computer generated and the data stored for research purposes. Frequent telephone conversations, site visits and constant attention to the reported information ensures high quality information. Both contracted laboratories are inspected by the Center for Disease Control, Atlanta, Georgia and approved as well. The culture data are used both for patient management and as a basis for various other projects.

Major Findings:

An average of 1,342 samples per month were processed by the contracting laboratory (Hazleton Laboratories) for recovery and final identification of most microorganisms recovered during 1972. Approximately 160 per month were those from the Leukemia Service, NCI, NIH and includes extensive anaerobic culturing of both environmental and patient surveillance cultures. The other approximately 1,184 cultures each month were from the Medical Service, BCRC, DCT, NCI.

Of 531 samples for viral isolation during a nine month period, the results of which have been completed, 54 (10%) were positive for a virus. The most frequent isolate was Herpes simplex virus, 36, followed by Cytomegalo virus (CMV), 9. A large proportion (60.4%) of cultures were derived from a small number of patients (14.6%) who were routinely screened for viruses. The number of specimens processed increased 17% over that of the previous year. During the same time interval 443 serum specimens derived from 199 patients were screened for viral antibody titer. Ninety-eight patients had more than one sera examined and 25.5% of them showed significant antibody titer rises. Half of these significant titers were against CMV.

This program continues to use outside contracted facilities for accurate and current microbiologic laboratory information for daily patient care. The use of computerized reporting, storage and retreival has facilitated reporting of data and its use in clinical care and research. The Contractors have responded to indicated needs by developing a flow chart and gas chromatographic analysis of volatile and nonvolatile fatty acids to aid in the identification of anaerobes, and by demonstrating that it is possible to prepare high titer anti serum against selected prevelant anaerobic

species to shorten time necessary for identification. They further established regression curves for organisms other than Pseudomonas aeruginosa against carbenicillin, one of the antibiotics used in the carbenicillin-gentamicin-cephalothin protocol used at BCRC. More rapid methods for viral identification are being investigated at the Viral Diagnostic Laboratory. Strains of microorganisms recovered by the contracted laboratories and returned to BCRC continue to provide the basis for a number of research projects.

Significance to Bio-medical Research:

The successful operation of contracted bacteriologic and viral services for use in direct patient care provides support for the cancer patients receiving chemotherapeutic agents. It provides the clinician with the immediate information needed in care of the patient, keeps him abreast of developing resistance to antibiotics in use, warns of cross contamination, and for patients with lowered host defense, provides data concerning the bacterial flora and antibiotic susceptibility prior to development of infection. The data generated by the program also provided the base for a number of research investigations.

Proposed Course:

To continue the program along the same lines.

Honors and Awards: None

Presentations:

A Laminar Air Flow Room During Use as a Protective Environment for Acute Leukemia Patients Undergoing Chemotherapy. Viola M. Young, Stephen C. Schimpff, William Greene, Norman Friedman, presented by Viola M. Young at the 72nd Annual Meeting, American Society for Microbiology in Philadelphia, April 1972.

Publications: None

Serial No.: NCI-6039
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Computerized Microbiology Reporting and Patient Information System for use by a Distantly Located Contracted Laboratory Facility

Previous Serial Number: Same

Principal Investigator: Gerald D. Vermeulen, M.D.

Other Investigators: Viola Mae Young, Ph.D., Robert Esterhay, M.D., Richard K.C. Hsieh, Dr. PH., John W. Gerster, William L. Robinson, Ralph F. Wachter and W. H. Greene, M.D.

Cooperating Units: Medical Service, NCI, BCRC; Health Services Research, U.S. Public Health Service Hospital, Balto., Md. 21211

Man Years (July 1, 1972 - June 30, 1973)

Total: 3
Professional: 1 1/2
Other: 1 1/2

Project Description:

Objectives:

1. To establish a practical method of recording and reporting by computer clinical microbiologic laboratory data generated by a distantly located laboratory facility and utilized by clinicians for patient support.

2. To provide a means of automating various clinical tasks made necessary by the use of contracted laboratory facilities and other clinical functions necessary in the operation of a clinical microbiology laboratory.

3. To provide a means of permanently recording BCRC patient and microbiologic data for use by clinicians and microbiologists to perform prospective and retrospective studies and to serve as a basis for quality control.

Methods Employed

Patients' names, hospital numbers, dates, samples, and requests are entered from the hospital into data files stored on Tymshare timesharing computer system. This information is immediately available to the distant contracting laboratory which then enters laboratory results as they become available. The new data is received twice daily by the hospital for clinical use. As each report becomes final it is transferred to a separate file and batch processed at the hospital computer center at which time the final report is produced. This data is permanently stored on mass storage disc and is used as the data base for data retrieval.

Major Findings

The patient census program (see Project Report NCI-6220) which records patient vital statistics, rooms, beds, attending physician, diagnosis, etc., is used by the Microbiology Reporting System to detect errors in hospital numbers, names, etc. It also serves as a base for epidermiologic reports of various pathogenic microorganisms and other data summaries.

The microbiology preliminary reporting system is still under development. Problems encountered, due to the time-sharing facilities being temporarily inoperable or overloaded, resulted in heavily loaded files. This has made it necessary to review the system to increase its speed and efficiency. Its operation has enhanced quality control and made it possible to currently review dangerous pathogens, development of resistant strains and to trace locations of patient carriers and their attending physicians.

The Microbiology final report generating, data storage and retrieval system continues to operate efficiently. The retrieval programs provide for retrieval by multiple parameters for use in patient care as well as for various research purposes. The data bank contains all of the patient data generated since July, 1968, when the first microbiology contract was instituted and all of the surveillance culture data for both patients and environment of the Laminar Air Flow rooms.

Significance to Bio-medical Research:

The census program enables the contracted laboratory to obtain current patient information necessary to the optimal processing of specimens. This program, together with microbiologic data, allows study of the transmission of specific microorganisms within the hospital.

The microbiology preliminary reporting system provides a means of keeping preliminary information on bacterial culture results at hand for the clinician's use. It allows immediate review of all current information on cultures of an individual patient or of all of a particular species of

organism present in patients or in the environment of the Laminar Air Flow rooms. The system also expedites the transfer of the data from the culture reports as they become final to magnetic tape for batch processing and storage at the hospital Computer Center.

The microbiology data storage and retrieval system generates the final report of the microbiology culture results for placement in the patient's chart, processes the data through a data screening and error detection program which produces an error report and a report of unusual results for review by the chief microbiologist, produces data summaries inclusive of any desired information, produces infection and antibiotic susceptibility reports and other data summaries as needed.

Proposed Course:

The microbiology preliminary reporting system will be modified to simplify and improve its operation. Other developments will include data summaries to be provided on a weekly and/or monthly basis to assist the Project Officer in management of the contract and to keep current information of important pathogens.

The microbiology final report generating, storage and retrieval system will continue to operate as at present.

Honors and Awards: None

Presentations:

Census: A remote-access Patient Information Storage and Retrieval System. William L. Robinson and Robert J. Esterhay, Jr, presented at the 10th Symposium on Biomathematics and Computer Science in the Life Sciences in Houston, March 1973.

Publications: None

Serial No.: NCI-6219
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies Directed Toward Elucidation of Cancer Patient Microbial Flora, Colonization by Specific Bacterial Genera and Infection

I. The Detection of Pneumocystis carinii Infection in Cancer Patients

II. An Investigation of Klebsiella spp. Isolated from Cancer Patients, their Serotype and Antibiograms

III. A Study of the Incidence and Types of Gram Negative Rods and other Pathogenic Microorganisms in the Upper Respiratory Tract of Cancer Patients

IV. Broad Spectrum Bacterial Antibody Evaluation of Cancer Patient Sera

V. Studies of New or Unusual Organisms Recovered from Cancer Patients

VI. Incidence of Antibodies against Heterogenetic Enterobacterial Antigen in Typhoid Vaccinated Subjects

VII. A Study of Proteus spp. Colonization and Clinical Significance in Cancer Patients

VIII. Anaerobes and their Effect in Cancer Patients

IX. Contamination of Products Used in the Care of Cancer Patients

X. Protective Environment for the Support of Cancer Patients Receiving Chemotherapy

XI. The Relationship of Bacterial Flora Recovered During the First Two Weeks of Hospitalization versus Hospital Acquired Microorganisms to Subsequent Infection

Serial No.: NCI-6219

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., I. J. Abramson, Ph.D., Robert C. Good, Ph.D., Norman R. Friedman, B.S., Dolores M. Kenton, B.S., Joseph G. Ouslander, Lemuel A. Moye III

Cooperating Units: Medical Service, NCI, BCRC, Hazleton Laboratories, Vienna, Virginia, Department of Biology, Johns Hopkins University.

Man Years (July 1, 1972 - June 30, 1973)

Total: 2
Professional: 1-1/2
Other: 1/2

Project Description:

Objectives:

The objective of this project is to develop information concerning the microbial flora of cancer patients, i.e., source, colonization, infection and response to infection. More specifically the objectives for each area are as follows:

I. To develop a diagnostic test for infection with Pneumocystis carinii to obviate the necessity of using lung biopsy as the only reliable diagnostic tool.

II. To serologically identify Klebsiella strains by means of the capsular swelling test to study their source, colonization and subsequent infection and to compare serological results with the antibiograms.

III. To determine the species and incidence of gram negative rods and other organisms in the upper respiratory tract of cancer patients and to determine which led to subsequent infection.

IV. To determine "normal" antibody levels in cancer patients as they enter the hospital to a variety of antigens prepared from micro-organisms which most frequently cause infection, to monitor changes in antibody titer during their hospital stay and as infection occurs.

V. To study new or unusual organisms recovered from cancer patients, determine their most probable classification, establish their antibiotic susceptibility and potential pathogenicity for the patient.

VI. To determine the possible antibody response of typhoid vaccinated patients to common antigen in view of the possibility that such antibodies might protect against the broad spectrum of gram negative rods which carry common antigen.

VII. To develop a system for detecting specific strains of Proteus and test a number of Proteus strains recovered from cancer patients to determine possible transmission from patient to patient, colonization and clinical significance.

VIII. To determine the possible role of anaerobes in infection of cancer patients utilizing the most fastidious techniques and compare the results with those obtained by the usual routine techniques.

IX. To determine the presence of microorganisms in such products as blood and blood products, food supplements, medications and cosmetics used by cancer patients.

X. To evaluate the use of Laminar Air Flow rooms and other air filtration systems to provide an environment free from exogenous microorganisms for cancer patients during periods of immunosuppression.

XI. To determine the infectious role of microorganisms endogenous to the cancer patient for the first two weeks of their hospital stay as compared to flora acquired from the hospital environment.

Methods Employed

Pneumocystis carinii antigens prepared from infected rats are being used in various serological techniques to achieve a means of detecting infection.

Biochemical tests are used to classify the Klebsiella spp. recovered from the patient samples and Difco Laboratories antisera are utilized for the capsular swelling test to identify particular strains of organism.

Retrieval programs for patient data stored on magnetic disk are being utilized to determine the number of patients in whom the normal flora of the upper respiratory tract is altered to include gram negative rods usually considered "enteric" and subsequent infection by these organisms.

Standard Microtiter hemagglutination test and other serological techniques are being utilized to determine serum antibody levels against various pathogens as well as to common antigens.

The method described by Craddock-Watson to test the Proteus group for bacteriocin production was modified to facilitate testing of a large number of specimens. This was accomplished by designing a multiple inoculation system which is capable of inoculating 24 producer broths simultaneously. Over 1000 clinical isolates collected over a 16 month period from 170 patients are being tested by their reaction to standard producers and by their bacteriocin productions as detected by standard indicators. Phage typing and the Dienes phenomenon will be used to further classify the Proteus strains. Results will be analyzed in terms of clinical significance and the relationship between the techniques utilized.

The strict anaerobic techniques devised by Hungate and later revised and empolyed by the Anaerobe Laboratories at the Virginia Polytechnic Institute are being used to study lesions and body fluids of the cancer patients to determine the possible role such anaerobic organisms may have in their infections.

Blood, blood products, medications, food supplements, cosmetics and other products used for or by patients are cultured according to standard aerobic and anaerobic microbiological techniques to determine the presence of microorganisms.

Surveillance cultures of Laminar Air Flow (LAF) rooms and the patients on the LAF protocol both inside and outside of the LAF rooms are performed according to standard microbiological procedures. Filtration units manufactured by the Med-Assist Corporation to be placed at the head and foot of hospital beds to produce sterile air flowing in a laminar direction over the bed-ridden patient are also being evaluated as a protective environment. Surveillance cultures of the room are taken before the patient's entry into the room, after entry and before the filters are turned on, after the filters are turned on without additional sterile procedures and after the filters are turned on with complete isolation procedures in effect. Surveillance cultures of the patient are taken bi-weekly. All cultures are performed according to standard microbiologic procedures.

Retrieval programs are being used to review the bacterial flora recovered from the patient during the first two weeks of hospitalization and that acquired by the patient from the hospital environment. The transient and resident strains will be analyzed and the relationship of this flora to microorganisms causing infection established.

Major Findings:

Gel diffusion studies with the Pneumocystis carinii antigen gave no reaction with the sera from rats drawn from the animals in which the antigen was produced. Effort is under way to obtain high titer antiserum.

Klebsiella strains of more than one capsular type may be harbored by a patient at different body sites or may change over time. Antibiotic susceptibility patterns do not correlate with capsular types of Klebsiella. Both Klebsiella oxytoca and Klebsiella ozaenae develop capsular swelling with various Klebsiella pneumoniae antisera.

Gram negative bacilli, including those generally considered to be "enteric" constitute a major portion of the flora of the upper respiratory tract of many debilitated patients. Of a total of 169 episodes of pneumonia at BCRC during 19 months, 66 were caused by such gram negative bacilli, which occurred either alone (36 cases) or in combination with other organisms (30 cases). K. pneumoniae was the most frequent causative agent (25 cases), Escherichia coli was the cause of 12, Enterobacter spp. of 9, Proteus mirabilis of 2, Pseudomonas aeruginosa of 19, Pseudomonas multivorans of 2 and Pseudomonas putida of 1. Many of the gram negative bacilli which became part of the resident flora of the upper respiratory tract prior to causing pneumonia were acquired from the hospital environment. Gram negative bacillary pneumonias were frequently associated with bacteremia and fatal in 58 of the 66 cases.

Of a total of 29 patients who had Escherichia coli bacteremia 38% were caused by E. coli serotype 06. Serotypes 03, 020, 023, 066, 0129 also caused bacteremias. Forty-five per cent of the E. coli strains causing bacteremia were untypable. In only one patient was a high hemagglutin response detected.

A pentose-fermenting gram negative rod which colonized a cancer patient for over two months has been studied by assimilation techniques to determine its ability to utilize unusual chemical compounds. It is non-pathogenic for mice.

Results of the bacteriocin and phage typing have not as yet been analyzed, but it is apparent that particular bacteriocin types do not parallel particular phage types and that phage typing may be a necessary and useful adjunct to further classify strains which fall within rather large bacteriocin groupings.

An "Anaerobile" has been fabricated which can be wheeled to the patient's bedside to enable the microbiologist to collect samples and inoculate them immediately onto anaerobic media under flowing gas thus allowing a minimum of exposure to oxygen. It also provides the physician with a laboratory cart on the ward where culturing of samples, both aerobically and anaerobically can be performed without recourse to the more distant laboratory.

Studies of blood platelets incubated at 37C and 26C revealed them to be contaminated with Staphylococcus epidermidis, Enterobacter cloacae and diphtheroids. Medications have been found to be contaminated with Bacillus spp. Food supplements have been found to be contaminated with Bacillus spp., Enterobacter cloacae, Lacto bacillus sp. and Clostridium subterminale. None of the supplements tested thus far have been free from such contamination. Yeasts have been recovered from talcum powder.

Surveillance culturing of the Laminar Air Flow rooms continues to show that if the patient is a Staphylococcus aureus carrier, this organism is often recovered from the floor while a Klebsiella pneumoniae carrier sheds this organism onto many surface areas as well as the floor. Water from the faucets is readily kept sterile, but other areas which contain water or moist surfaces, such as commodes, sinks, showers and drains, are difficult to keep free of microorganisms and often contain Pseudomonas spp. Skin flora from the patient is often found in small numbers on various surfaces within the environment. The air within the LAF units is relatively free from bacteria.

The study performed with the Med-Assist Corporation filtration units show them to be able to produce a laminar flow of sterile filtered air over the patient while lying in the bed. Additional sterile procedures are necessary to reduce contamination in the rest of the room.

Analysis of microbiological cultures of cancer patients over a 4 year period revealed that of the Klebsiella spp. and Enterobacter spp. recovered from 1014 sites, K. pneumoniae compised 44% of the cultures, K. ozaenae 4%, E. cloacae 26%, E. aerogenes 16% and E. liquefaciens 2%. Of the patients whose primary surveillance cultures of more than one body area during the first two weeks of hospitalization yielded K. pneumoniae 21.2% became colonized with this organism and developed infection; 14.7% of patients similarly carrying E. aerogenes became infected. If K. pneumoniae was recovered from the upper respiratory tract only during the first two weeks of hospitalization 7.3% of 96 patients became colonized and infected, from the urogenital and rectal areas, of 229 patients, 11.4% became colonized and infected. E. aerogenes gave similar infection rates though the total number of patients involved was less and 18.7% of the 16 patients whose skin was the only recovery site during the first two weeks of hospital stay, developed infection. If the Klebsiella-Enterobacter Group of organisms was recovered from primary cultures taken from multiple sites, in approximately 50% of the patients this bacterial group became resident flora, whereas approximately 21% of the patients with Klebsiella-Enterobacter Group grown from the respiratory tract or urogenital-rectal areas became colonized.

Significance to Bio-medical Research:

All of these studies have helped to define the species of microorganisms which colonize patients, the location of patient sites which are colonized, and enables a better understanding of infection in the patient as well as the patient's response to that infection.

Proposed Course

To continue along the lines indicated.

Honors and Awards:

1. The "Anaerobile" has been selected by the Kontes Glass Company for manufacture. It will be exibited at the Federation of American Societies of Experimental Biology meeting in Atlantic City, April 1973 and at the 73rd Annual Meeting of the American Society of Microbiology in Miami Beach, May, 1973.

2. Presentation No. 2 (below) chosen for press release at the 73rd Annual Meeting of the American Society of Microbiology, Miami Beach, May, 1973.

Presentations:

The Presence of Enterobacter and Klebsiella spp. in Cancer Patients and their Subsequent Role as Pathogens. M.R. Moody, V.M. Young, G.D. Vermeulen, J.W. Gerster, presented by Marcia R. Moody at the 73rd Annual Meeting of the American Society of Microbiology in Miami Beach, May, 1973.

Gram Negative Rod Flora in the Upper Respiratory Tract of Cancer Patients. Their Role in Pneumonia. V.M. Young, E.A. Sickles, M.R. Moody, P.H. Wiernik, presented by V.M. Young at the 73rd Annual Meeting of the American Society of Microbiology in Miami Beach, May, 1973.

Anaerobic Susceptibility Testing of Anaerobes Using Disc and Broth Techniques, presented by I.J. Abramson to the Anaerobic Section of Infectious Diseases, Johns Hopkins University, April 6, 1973.

Anaerobes, a series of lectures given by I.J. Abramson to the Medical Technology students of the US PHS Hospital, January, 1973.

Publications:

Buchholz, D.H., Young, V.M., Friedman, N.R., Reilly, J.A., and Mardiney, M.R.: Detection and Quantitation of Bacteria in Platelet Products Stored at Ambient Temperature. Accepted for publication by Blood, 1973.

Serial No.: NCI-6219

Nelson, R.M., Reed, J.R., and Kenton, D.M.: Microbiological Evaluation of Decontamination Procedures for Hydrotherapy Tanks. Accepted for publication by Physical Therapy, 1972

Serial No.: NCI-6223
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Pseudomonas aeruginosa in a Cancer Research Center

I. Comparison of Immunotypes from Cancer and non-Cancer Patients

II. Hemagglutinin Levels to Pseudomonas aeruginosa in Cancer Patients

III. Incidence of Pseudomonas species other than P. aeruginosa in Cancer Patients

IV. Relationship of Pseudomonas aeruginosa Immunotypes to Antibiotic Sensitivity Patterns

V. Phagocytosis of Pseudomonas aeruginosa by Immune and Non-immune Guinea Pig Macrophages

VI. Influence of Genetic factors on the Emergence of Carbenicillin and Gentamicin Resistant Pseudomonas aeruginosa.

VII. The Effect of Controlled Environmental and Antibiotic Regimens on the Induction of Atypical Pseudomonas aeruginosa in Laminar Air Flow Room Patients

VIII. The Effect of Immunosuppressive Drugs and Pseudomonas aeruginosa on the Potientiation of Infection in the Compromised Host

Previous Serial Number: Same

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D., W. H. Greene, M.D., Dolores M. Kenton, B.S., Myron Fisher, Ph.D.

Cooperating Units: Parke Davis and Co., Detroit, Michigan

Man Years (July 1, 1972 - June 30, 1973)

Total: 2
Professional: 1-1/2
Other: 1/2

Serial No.: NCI-6223

Project Description:

Objectives:

Pseudomonas aeruginosa is the most frequent cause of infectious death in patients receiving chemotherapy for acute myelocytic leukemia and a frequent cause of infection in other immunosuppressed cancer patients. The main objectives of this project are to compare the distribution of Pseudomonas immunotypes recovered from cancer patients with those from non-cancer patients to determine the modes by which P. aeruginosa immunotypes develop resistance to antimicrobial agents; to establish the effects of immunosuppressive therapy on the pathogenesis of P. aeruginosa in immunologically compromised cancer patients and the patient's response to this immunogenic stimulus. Recovery of other Pseudomonas spp. is to be related to colonization and/or infection of the patients and their antibiotic susceptibilities determined.

Methods Employed:

All Pseudomonas strains recovered from the patients and their environment were speciated and all P. aeruginosa strains were immunotyped using antisera supplied by M. Fisher of Parke, Davis and Co. Hemagglutinin levels of patient antibodies were determined by the microtiter technique. Antimicrobial susceptibility tests were performed by the Bauer-Kirby-Sherris-Turck technique and antibiotic minimal inhibitory concentrations were established by use of an agar plate dilution technique. Pseudomonas strains other than P. aeruginosa recovered from cancer patients were speciated by a schemata derived from the systems of Stanier (minimal medium), Hugh (oxidative-fermentative (O-F reactions) and Pickett (O-F reactions utilizing buffered media). Macrophages from guinea-pigs were incubated with an immunizing serotype of P. aeruginosa and the phagocytic endex determined. Carbenicillin and gentamicin resistant strains of P. aeruginosa were investigated for the presence of "R" factors by mating with E. coli K-12 which is susceptibel to both agents and by "curing" the strains of the R factor with acridine orange and sodium dodecyl sulface. Other resistant gram negative microorganisms which were recovered concurrently with susceptible strains of P. aeruginosa from the same site were mated with the latter strains; after exposure to sublethal concentrations of the antibiotics, the similarity between the original resistant P. aeruginosa strain and the new resistant strain was determined by biochemical, physical and serological methods. Mice and guinea pigs were inoculated with cytoxan and P. aeruginosa simultaneously; potientiation of infection and/or lethality was determined by comparison to groups of animal which received the bacterium or the immunosuppressive drug alone.

Major Findings:

This study has revealed that P. aeruginosa type 1, a type prone to transitory colonization in patients, is more prevalent in noncancer patients, while types 4 and 7, which, in a previous study, were recovered from the blood in approximately one-fourth of patients colonized with these strains, are more common in cancer patients. The highest incidence of P. aeruginosa among cancer patients occurred in ALL (55%) and ANLL (56%) whereas the lowest was in Hodgkins (10.3%) and lymphosarcoma (11.8%). Types 2, 6, and 7 were recovered with approximately equal frequencies. Type 3 was more frequently recovered from lymphoma-myeloma patients but very rarely from acute leukemic patients. The highest incidence of types 4 and 5 occurred in acute leukemia and solid tumor patients respectively. Increasing numbers of carbenicillin and/or gentamicin resistant strains of P. aeruginosa have been isolated from cancer patients; susceptible strains of the same type were present originally. Preliminary data would seem to suggest the transfer of "R" factors from other resistant gram negative bacteria which are present in the site concurrently to susceptible strains of P. aeruginosa and the induction of resistant strains when carbenicillin-gentamicin therapy is instituted. Similarly, P. aeruginosa strains which were resistant to these agents have been recovered from Laminar Air Flow unit patients; they, too, were preceded by susceptible strains of the same type. However, these resistant strains are atypical in growth, temperature requirements, pigment production, and serological characteristics. They are susceptible only to polymixin B and colistin, always belong to serotype 1 or 2, and tend to colonize the gastrointestinal tract. The episomal transfer of "R" factors does not appear to be involved in the emergence of these atypical resistant strains, as the resistance is not elminiated by acridine orange. Pseudomonas spp. other than P. aeruginosa have been recovered from these patients; P. multivorans (cepacia) and P. maltophilia are the most common.

P. multivorans had a predilection for the urinary tract whereas P. maltophilia was more common in the upper respiratory tract. P. multivorans was recovered at autopsy from three patients and all three patients were colonized prior to death (only one patient had a positive blood culture). Conversely, P. maltophilia was recovered at autopsy from four patients who were not colonized prior to death (only one patient had had a positive blood culture). Fifteen percent of patients with P. fluorescens had bacteremias but this organism was recovered at autopsy only once (no prior isolation). Other species were found much less frequently except in the environment. P. multivorans was resistant to most antibiotics including to Oramycin. However, it was susceptible to very low concentrations of trimethoprim ($\leq$ 1 ug/ml) and the susceptibility has proven to be a stable genetic marker for this Pseudomonas sp. It was resistant to sulfamethoxazole but when combined with trimethoprim, it was susceptible to 8-16 ug/ml. Studies of the development of "tolerance or unresponsiveness" to P. aeruginosa as an immunogen when a specific type is introduced to the host who is concurrently undergoing immunosuppressive therapy and its effect on infection are in progress.

Significance to Bio-medical Research:

These studies have led to a greater understanding of the dynamics of interaction between Pseudomonas aeruginosa in the environment and in the patient. They have made it possible to define related flora and to prepare for a potentially dangerous ecologic situation wherein resistant organisms have colonized a number of susceptible patients. They have also allowed us to predict the likelihood of severe infection in certain patient groups and to better understand the mechanism of the disease produced.

Proposed Course:

I. The work is completed and a publication is in preparation.

II. More patient sera have been collected. Testing for determination of hemagglutinens by the automated microtiter system has begun. Data are too preliminary for conclusions. Serologic studies will be continued.

III. This project is completed. One segment of the study has been published and another publication is in preparation.

IV. This project has been completed, but studies of P. aeruginosa susceptibility patterns are continuing as resistant strains have emerged in our patient population.

V. This study will not be continued along the previously indicated lines as products elaborated by this microorganism cause widespread destruction of the macrophages and accurate phagocytic indices could not be performed. Other methods are to be investigated.

VI. This project will continue along the lines indicated.

VII. An investigation of the observed phenomenon is now under way.

VIII. Animal studies are currently in progress.

Honors and Awards: None

Presentations:

The Emergence of Carbenicillin and Gentamicin Resistant Pseudomonas aeruginosa: W.S. Green, M.R. Moody, S.C. Schimpff, V.M. Young and P.H. Wiernik. Presented by W.H. Greene at the Eastern Section of American Federation of Clinical Research, Boston, Mass. 1973 (also listed under NCI-6266)

Serial No.: NCI-6223

Pseudomonas Septicemia: Incidence, Epidemiology, Prevention, and Therapy in Patients with Advanced Cancer: S.C. Schimpff, W.H. Greene, V.M. Young, and P.H. Wiernik. Presented by S.C. Schimpff at the Institute Jules Bordet, Brussels, Belgium, January 1973.

Publications:

In vitro Antibiotic Susceptibility of Pseudomonads other than P. aeruginosa Recovered from Cancer Patients. Antimicrobial Ag. Chemotherapy 2:344-349, 1972. Moody, M.R., Young, V.M., and Kenton, D.M.

Origin of Infection in Acute Nonlymphocytic Leukemia. Significance of Hospital Acquisition of Potential Pathogens. Ann. Intern. Med. 77:707-714, 1972. Schimpff, S.C., Young, V.M., Greene, W.H., Vermeulen, G.D., Moody, M.R. and Wiernik, P.H. (also listed under NCI-6264).

Serial No.: NCI-6254
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Research Activities Directed Toward Improvement of Microbial Laboratory Techniques

I. A comparison of Buffered Glycerol Saline, Todd-Hewitt Broth and Unpreserved Fecal Material as the Means of Sample Collection to Determine Fecal Microbial Flora

II. A Study of Relative Effectiveness of Different Types of Blood Culture Media and Laboratory Techniques for the Isolation of Specific Types of Microorganisms

III. Development of a Multitube Micromethod for Identification of Alkaline-Producing Gram Negative Rods

IV. Studies of Antibiotic Susceptibilities Involving New Methodology or Newly Developed Antibacterial or Antifungal Agents.

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Gerald D. Vermeulen, M.D.
I. J. Abramson, Ph.D., Kevin Connelly

Cooperating Units: Hazleton Laboratories, Vienna, Virginia

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/3
Professional: 3/12
Other: 1/12

Project Description:

Objectives:

To determine which of several methods of preservation would allow recovery of a majority of the microorganisms present in fecal material. To determine the best combination of culture media for recovery of microorganisms from blood. To develop a simple, rapid system for classification of the alkaline-

producing gram negative rods which are abundant in the environment, important to patient care and difficult to speciate. Characteristic of this group is their lack of enzymes to ferment the commonly utilized sugars. To set up standards for susceptibility testing of new antibiotics, determine blood and/or urine levels and establish the susceptibility of selected microbial isolates.

Methods Employed:

Todd-Hewitt Broth, buffered glycerol saline and unpreserved fecal material were compared for their ability to retain viability of fecal organisms during transport to the laboratory. Tryptic soy broth (Difco), brain heart infusion broth with 30% sucrose and 0.1% agar (Difco), thiol (Difco), brain heart infusion broth with cysteine under nitrogen and thioglycollate broth are under comparison for their ability to support growth of organisms from blood. An attempt is in progress to utilize plastic strips holding minaturized compartments which contain the substrate.

To determine the appropriate disc concentrations and zone diameters of carbenicillin to be used for microorganisms other than P. aeruginosa in the Bauer-Kirby antibiotic susceptibility determinations, discs were first prepared containing 5, 10, 15, 22, 33, 50 and 100 micrograms of carbenicillin. Zone diameters were measured to the nearest millimeter with callipers and results compared with minimum inhibitory concentrations determined by the microtitration procedure. Regression curves were drawn from Staphylococcus aureus, Group D Streptococcus, Klebsiella pneumoniae and Escherichia coli. Various yeasts recovered from autopsy samples were tested for growth-inhibition by 5-Fluorocytosine in broth dilution tests in an effort to determine which yeasts involved in pathogenic processes were susceptible to the drug.

Major Findings:

A study of the recovery rates of fecal organisms achieved using rectal swab samples preserved in Todd-Hewitt broth and buffered glycerol saline from the rectum and perirectal skin as compared to unpreserved stool showed that Staphylococcus aureus recovery rates were greatest from the unpreserved stool. However, gram negative rods were more frequently recovered from rectal swab samples preserved in Todd-Hewitt broth. Seventy per cent of Klebsiella spp. and 92% of Proteus morganii strains were recovered using this sampling method. None of the other three techniques yielded recovery rates superior to samples preserved in Todd-Hewitt broth (p=.05) and this method had an overall recovery rate of 88% for gram negative rods.

More recent data on the recovery rate of microorganisms from various media is presently being evaluated and compared with data accumulated previously.

Insufficient data on the multitude micromethod for identification of alkaline-producing gram negative rods has been accumulated to determine its practicability.

Isolates of S. aureus, Group D Streptococcus, K. pneumoniae and P. aeruginosa fell into populations that were susceptible and resistant to carbenicillin while all E. coli isolates appeared to be susceptible. From the preliminary data it would appear that while the selection of the 50 mcg. disc was appropriate for testing of P. aeruginosa (gave separate populations), the 10 mcg. disc would be more useful for measuring susceptibility of S. aureus, Group D Streptococcus, E. coli and possibly K. pneumoniae. Since few of the latter were susceptible to carbenicillin, the statistical evaluation was subject to significant error.

Of 30 strains of Candida albicans recovered from autopsy samples, approximately half were inhibited by 5-Fluorocytosine. Four strains of Candida parapsiloris and two strains of Candida krusei were resistant, while three strains of Cryptococcus neoformans, four Tosulopses globrata and one Candida tropicalis were inhibited. In no instance were the organisms killed; the activity was inhibitory.

Significance to Bio-medical Research:

Optimal bacterial culture techniques are essential to determining the bacterial flora of the patient and his environment, to recovering the causative agent of infections and to assessing the optimal antibiotic treatment.

Proposed Course:

I and II. These studies are completed and the results being prepared for publication.

III. To continue along the lines indicated.

IV. Additional isolates, particularly of the Enterobacter spp. will be examined to further determine optimal carbenicillin disc concentrations. Relationships between the minimum inhibitory concentrations and agar diffusion results will be examined when optimal carbenicillin disc concentrations have been established.

Additional yeasts will be tested for their inhibition by 5-Fluorocytosine and a determination reached as to whether this drug would have value in therapy of yeast infections in those cases when the yeast is inhibited.

Honors and Awards: None

Presentations: Have been invited to present the data on carbenicillin at the FDA, but date has not been set as yet.

Publications:

Culturing of Anaerobic and Aerobic Microorganisms Using a Mobile Unit (Anaerobile) in a Nosocomial Environment. Abramson, I. J. (to be submitted for publication).

Synergism of Antibiotic Combinations Against Treponemes (1972), Abramson, I.J. and R.M. Smibert. Brit. J. Ven. Dis. 48:113-115.

Serial No. NCI-6217
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Prevention of Infections in Patients with Cancer Including Evaluation of Protected Environments and Surveillance Cultures

Previous Serial Number: 6265, 6217, 6029

Principal Investigator: William H. Greene, M.D.

Other Investigators: Viola Mae Young, Ph.D., Stephen C. Schimpff, M.D., Clarence L. Fortner, M.S., Lois Jepsen, R.N., Peter H. Wiernik, M.D., Michael D. Walker, M.D., Dennis Duda, M.Ed., Nancy Cusack, I.J. Abramson, Ph.D., Norman Friedman, B.S., Dolores Kenton, B.S., Marcia Moody, M.S.

Cooperating Units: Research Microbiology Section, BCRC: Center for Disease Control, Atlanta, Georgia; Hazleton Laboratories, Vienna, Virginia

Man Years

Total:	7
Professional:	4 1/2
Other:	2 1/2

Project Description:

Objectives:

Infection is the most common cause of death in patients with acute leukemia and is a major cause of death in all other types of cancer, especially those receiving cancer chemotherapy. At this Center, the most common cause of infectious death is Pseudomonas aeruginosa. As noted in another project (6264), approximately half of the microbiologically documented infections in patients with acute leukemia were due to organisms which had been acquired following admission to the hospital. Again, the most prevalent cause of infection was Pseudomonas aeruginosa. Therefore, various avenues have been pursued in an attempt to reduce the acquisition of hospital organisms and to prevent infection by the patient's endogenous flora.

A) Protected Environment with the Use of Laminar Air Flow Rooms and/or Oral, Nonabsorbable Antibiotics - All adult, non-infected patients with acute nonlymphocytic leukemia are randomized into one of the following three groups: 1) laminar air flow unit plus ingestion of oral, nonabsorbable antibiotics, 2) ward care with oral, nonabsorbable antibiotics, or 3) ward care without

Objectives (cont'd):

antibiotics. Patients in the three groups are all treated identically with respect to nursing care, physician care, and supportive care. Patients in the laminar air flow room are treated under sterile conditions, using sterile supplies, and a cooked food diet. All infections are carefully observed and recorded.

B) Studies to Determine the Routes of Hospital Acquisition of Potential Pathogens - Cultures of multiple sites in the hospital environment are taken by the Research Microbiology Laboratory, with emphasis on water sources such as ice machines, respirators, sinks, and sink drains. Cultures of physicians', nurses', and technicians' hands are taken at intervals. Careful observations of handwashing techniques, sterilization techniques, food preparation, and patient care are in progress. All Pseudomonas strains are speciated and serotyped (Fisher method), Klebsiella spp. are serotyped, Staphylococcus aureus are phage typed, and some Proteus spp. are proticine and Dienes typed. This data is correlated in an attempt to determine how and from where a cancer patient acquires potential pathogens from the hospital environment.

Major Findings:

A) Laminar Air Flow Rooms - As of 3/31/73, 55 patients with acute nonlymphocytic leukemia have been eligible for randomization to the laminar air flow room protocol - of whom 21 were treated within the LAF room, taking oral nonabsorbable antibiotics; 16 on the ward taking antibiotics and 18 on the ward without antibiotics. The mean and median ages of the three groups were comparable, as were the mean and median days on study and the percentage of patient days in each group with mild (<1000), moderate (<500) and marked (<100) granulocytopenia. Although not statistically significant in every category of response, there appears to be a biologically significant difference between the "laminar air flow" and"ward plus antibiotics" groups when compared with the "ward alone" group. In particular, there is a statistically significant increase in remission rate, with an associated decrease in death rate and, especially, infectious death rate among the former groups when compared with the latter. Moreover, the frequency of infectious episodes and of severe infections in the laminar air flow unit and ward plus antibiotics groups is one-half that seen in the ward alone group.

Because patients entered on the study remain "on study" until death or remission ensues, analysis of survival is relevant. Although little difference is seen at 30 days after entry into the study, at 60 days approximately two-thirds of all patients in the laminar air flow unit and ward plus antibiotics groups were alive, compared to 39% in the ward alone group. At 90 days, 62% in the former groups were alive compared to only 28% in the latter. Thus, from the point of view of delivery of adequate anti-leukemic therapy (defined

as three or more courses of anti-leukemic drugs), 70-79% of patients in the LAF and ward plus antibiotics groups were alive sufficient time to receive adequate therapy compared to 53% in the ward alone group.

As noted previously, Pseudomonas aeruginosa and Klebsiella sp. infections are considerably decreased by the ingestion of oral nonabsorbable antibiotics, as are pneumonias, anorectal infections and urinary tract infections. In contrast, nasopharyngeal, oropharyngeal and cutaneous infectious appear to be little affected by such manipulation.

Recent observations, in conjunction with part B of this project, have documented the appearance of carbenicillin and/or gentamicin-resistant Pseudomonas aeruginosa organisms of multiple serotypes among 14 patients. In large measure, development of gentamicin resistance (with or without carbenicillin resistance) could be temporally correlated with the use of oral gentamicin as part of this research protocol, whereas carbenicillin resistance (without associated gentamicin resistance) was due to the use of parenteral carbenicillin. Few clinically significant infections could be traced to gentamicin-resistant organisms, which seemed to be decreased in virulence, but those with carbenicillin resistance were quite virulent.

Although the data is approaching statistical significance, the study needs to be continued for the acquisition of further patients so as to be certain of the implications currently being suggested.

B) Studies to Determine the Routes of Hospital Acquisition of Potential Pathogens - In addition to previous random hand cultures of hospital personnel demonstrating 43 of 126 (34%) contaminated with either S. aureus or gram negative bacilli, a more recent survey of both employees' hands and hospital food revealed that 45 (11%) of 403 single-hand cultures and 11 (52.4%) of 21 meals cultured were contaminated by gram-negative bacilli, of which many were clinically significant serotypes of Pseudomonas aeruginosa and strains of Enterobacter, Klebsiella, and E. coli. This has led to extensive utilization of fast-breaking, bactericidal hand foams; use of isolation precautions for patients carrying resistant organisms; and institution of disposable gowns for nurses and physicians alike in the Tumor Intensive Care Unit.

Proposed Course: Continue the above two projects.

Significance to Cancer Research:

The two projects described herein are attempts at prophylaxis of significant bacterial infection among leukopenic patients being treated for advanced malignancy with aggressive chemotherapy. In view of the fact that infection is the leading cause of death among patients with acute leukemia, many of whom die prior to delivery of adequate anti-leukemic therapy, prophylaxis of infection, in the individual by use of LAF units and oral nonabsorbable antibiotics and in the population by attention to epidemiologic patterns of colonization

Significance to Cancer Research (cont'd):

and resistance, may itself contribute to higher response rates for standard chemotherapy or allow increased doses of chemotherapy with acceptable morbidity.

Honors and Awards: None

Publications:

1. Schimpff, S., Moody, M., Young, V.M.: Relationship of colonization with Pseudomonas aeruginosa to development of Pseudomonas bacteremia in cancer patients. Antimicrob Agents and Chemother 10:240-244, 1970.

2. Schimpff, S., Young, V.M., Greene, W., Vermeulen, G., Moody, M., Wiernik, P.: Origin of infection in acute nonlymphocytic leukemia - significance of hospital acquisition of potential pathogens. Ann Int Med 77:707-714, 1972.

Presentations:

I Dr. Greene

1. A controlled clinical trial of unidirectional air flow reverse isolation and oral antibiotic intestinal flora suppression in decreasing infection in the leukemic patient. Eastern Section of the American Federation for Clinical Research, Philadelphia, Pennsylvania, December, 1971.

2. The emergence of carbenicillin and gentamicin resistant Pseudomonas aeruginosa in a cancer center, Eastern Section of the American Federation for Clinical Research, Boston, Massachusetts, January, 1973 .

II Dr. Schimpff

1. Relationship of colonization with Pseudomonas aeruginosa to development of Pseudomonas bacteremia in cancer patients. 10th Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Illinois, October,1970.

2. Reverse isolation on a cancer service, Grand Rounds, D.C. General Hospital, Georgetown Medical Service, Washington, D.C., March, 1971.

3. Protected environment program at the BCRC. Second Annual Contractors Meeting, Annapolis, Maryland, October, 1971.

4. Laminar air flow units for patients with leukemia, Infectious Disease Seminar, Section of Infectious Diseases, University of Maryland School of Medicine, Baltimore, Maryland, December, 1971.

5. Laminar air flow units for patients with leukemia, Third Annual Contractors Meeting, Annapolis, Maryland, May, 1972.

Presentations (cont'd.):

6. Hospital-acquired pathogens and subsequent infection in patients with acute nonlymphocytic leukemia. 12th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, New Jersey, September, 1972.

7. Laminar air flow units - significance in acute leukemia patients, Tufts University, Boston, Massachusetts, December, 1972.

8. Pseudomonas species - incidence, epidemiology, prevention and therapy. Institute Jules Bordet, Brussels, Belgium, January, 1973.

9. Infections in cancer patients. Veterans Administration Hospital, Department of Infectious Diseases, Baltimore, Maryland, March, 1973.

III Dr. Young

1. A study of microorganisms recovered from a laminar air flow room during use as a protective environment for acute leukemia patients undergoing chemotherapy, Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania

2. The behavior of endogenous and exogenous gram negative rod flora in leukemia patients in an open ward and laminar air flow environments, 11th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, New Jersey,

3. Gram negative rod flora in the upper respiratory tract of cancer patients. Their role in pneumonia, 73rd Annual Meeting of the American Society for Microbiology, Miami, Florida, April, 1973.

IV Mrs. Moody

1. The presence of Enterobacter and Klebsiella spp. in cancer patients and their subsequent role as pathogens, 73rd Annual Meeting of the American Society for Microbiology, Miami, Florida, April, 1973.

Serial No.:NCI-6230
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease

Previous Serial Number: Same

Principal Investigators: Peter H. Wiernik, M.D.
Michael J. O'Connell, M.D.

Other Investigators: John Sutherland, M.D.
Robert Kirschner, M.D.
Arthur Abt, M.D.
Harold E. Ramsey, M.D.
Linell Murphy, M.D.

Cooperating Units: Pathology, Tumor Surgery, and Radiology Departments, USPHS Hospital

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives:

Accurate determination of extent of lymphomatous involvement with Hodgkin's Disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Major Findings:

Over 100 patients with Hodgkin's disease have undergone laparotomy and splenectomy for staging. The pre-operative assessment of splenic involvement was incorrect in over 1/3 of the patients studies. Many spleens of normal size were found to be involved and some large spleens were free of disease. Abdominal lymph node biopsies revealed that approximately 1/3 of the lymphangiograms

Major Findings: (Cont'd)

judged to be definitely abnormal or suspicious prior to laparotomy were in fact false positives. On the other hand, only 1 of 47 lymphangiograms felt to be entirely normal was shown to be false negative on the basis of lymph node biopsy.

Involvement of nodes in the porta hepatis was documented in 26% of the patients in whom nodes from this region were sampled, and mesenteric nodes were positive in 3.4% of the nodes sampled. Unsuspected hepatic involvement was documented in 2 cases and an additional 2 patients with liver involvement suspected on the basis of Ann Arbor criteria proved to have negative liver biopsies. Six patients have been found to have occult disease outside the usual inverted Y abdominal radiation therapy port who might have otherwise been considered candidates for total nodal radiation therapy using conventional radiotherapy ports.

There has been no operative mortality and minimal morbidity. Tolerance to extended field radiotherapy and combination chemotherapy may be improved as a result of splenectomy. There is no indication to date that splenectomy has adversely affected either the incidence or severity of infectious complications among this patient population.

Proposed Course:

Continue investigation.

Honors and Awards: None.

Publications:

1. Zarembok, I., Ramsey, H.E., Sutherland, J., and Serpick, A.A.:Laparotomy and Splenectomy in the Staging of Untreated Patients with Hodgkin's Disease. Radiology 102:673-678, 1972.

Presentations:

1. O'Connell, M.J., Sklansky, D.B., Greene, W., Abt, A., Kirschner, R., Ramsey, H., Murphy, L. and Wiernik, P.H.: Routine Staging Laparotomy in the Management of Hodgkin's Disease, National Meeting of the American Federation for Clinical Research, Atlantic City, N.J., April 29, 1973.
2. Wiernik, P.H., O'Connell, M.J., Abt, A., Kirschner, M.D., Murphy, W.L, Sutherland, J., and Sklansky, D.B.: Splenic Hodgkin's Disease (H.D.): Detection, Incidence, and Prognostic Significance. Presented at the Symposium on the Pathophysiology of the Spleen, Cluj, Romania, May 11, 1973 (Invited paper).
3. Wiernik, P.H.: The Case for Routine Laparotomy in the Staging of Hodgkin's Disease. Case Western Reserve University School of Medicine, February 18, 1973.

Serial No.: NCI-6259
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Extension of the Computerized Problem-Oriented Medical Information System (PROMIS) into the Cancer Treatment Area

Previous Serial Number: Same

Principal Investigator: Robert J. Esterhay, Jr., M.D.

Other Investigators: Paul Chang, M.D.
William R. Grove, Pharmacist
William L. Robinson, Research Programmer
Peter H. Wiernik, M.D.
Michael D. Walker, M.D.
Viola M. Young PhD
Sarah Chamberlain, R.N.
Clarence L. Fortner, Pharmacist

Cooperating Units: PROMIS Laboratory
Department of Medicine
University of Vermont
Burlington, Vermont 05401
Telephone: (802) 656-3946

Cooperating Investigators: Lawrence L. Weed, M.D.
Laura B. Weed, M.D.
George E. Nelson, M.D.
Jan R. Schultz, Research Associate
Stephen V. Cantrill, Research Programmer
Keith G. Morgan, Research Programmer

Man Years:

Total:	3
Professional:	3
Other:	0

Project Description:

Serial No.: NCI-6259

Objectives:

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device with electrostatic printer similar to those in use in Burlington, Vermont, where the computer is located. BCRC has been responsible for the installation and maintenance of this equipment and the telephone line to Burlington since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.

2. The basic components of the remote terminal at the BCRC are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, Digiscribe, and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

3. The BCRC staff has developed antineoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont as part of the PROMIS system. Currently over 20,000 displays have been developed by the PROMIS group.

4. Problem-specific display plans for acute non-lymphocytic leukemia have also been developed which includes a series of protocols for treating this specific problem. The protocol displays couple the user to the protocol specific information and ordering drug display sequences.

Major Findings:

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem defining sequence determines the specific plan, and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific antineoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user can couple learning with doing while gathering information and treating patients' cancer problems.

Significance to Cancer Research:

1. The PROMIS system provides a comprehensive approach to bringing current cancer knowledge to bear directly on the individual cancer patient's problems.

2. Explicit definition by structured cancer displays of logic and action pathways serves as a well-defined point of departure for discussion, feedback loops and correction of problem-specific plans (research protocols) for logic and direct care of the cancer patient.

3. Inherent in the PROMIS system but not necessarily a direct consequence of the project is the physician's ability to communicate to all others who have immediate care of the patient. All individuals who work with patients can quickly ascertain what the problems are and what is being done for them.

4. The PROMIS system is a prototype for a general system which will include cancer displays as an integral part of the system for patient care and medical education.

5. The expected date for full-scale implementation of PROMIS cannot be stated at this time.

Proposed Course:

Continued study.

1. Maintainence of the BCRC-PROMIS communication linkage by the communications programmer at the PROMIS Laboratory, Burlington, Vermont and the communications programmer at the BCRC, Baltimore, Maryland.

2. Development by the BCRC staff of problem specific cancer display plans for leukemia, lymphoma, brain tumors and selected solid tumors.

3. Audit of BCRC's developed cancer displays by the PROMIS staff for accuracy, completeness, and correctness of clinical judgement in both the content of the displays and their sequencing.

Honors and Awards: None

Publications: None

Presentations:

1. Esterhay, R.J., Jr., Wiernik, P.H. and Fortner, C.L., Tenth Symposium on Biomathematics and Computer Science in the Life Sciences, March, 1973, Houston, Texas. "Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System (PROMIS)"

Lectures: Robert J. Esterhay, Jr., M.D.

1. All Medical and Dental Officers and Chiefs of Departments, U.S. Public Health Service Hospital, Baltimore, Maryland, August, 1972, "Problem Oriented Medical Record"

2. Staff Meeting, Sacred Heart Hospital, Cumberland, Maryland, September, 1972, "Problem Oriented Charting"

3. Health Record Administration Program, Public Health Service, Baltimore, Maryland, October, 1972, "Computerized Problem-Oriented Medical Record"

4. Grand Rounds, Greater Baltimore Medical Center, Towson, Maryland, November, 1972, "Problem Oriented Grand Rounds"

5. Computers in Clinical Medicine, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland, February, 1973, "Computerized Problem-Oriented Medical Information System (PROMIS)"

6. Grand Rounds, Maryland General Hospital, Baltimore, Maryland, March, 1973, "Audit of the Problem Oriented Medical Record".

Serial No. NCI-6260
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation of the Efficacy and Toxicity of Daunomycin in a 180 mg/M^2 Single IV Injection in the Treatment of Acute Nonlymphocytic Leukemia

Previous Serial Number: Same

Principal Investigator: William H. Greene, M.D.

Other Investigators: David Huffman, M.D., Nicholas Bachur, Ph.D., M.D., Stephen Schimpff, M.D., Jerome Block, M.D., and Peter H. Wiernik, M.D.

Project Description: Study completed.

Serial No. NCI-6261
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Hyperalimentation in Patients with Malignant Disease

Previous Serial Number: Same

Principal Investigators: Clarence L. Fortner, M.S.,R.Ph., William H. Greene, M.D.

Other Investigators: Robert S. Benjamin, M.D., James C. Cradock, M.S., Lois Jepsen, R.N.

Project Description: Study discontinued.

Serial No.:NCI-6263
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert S. Benjamin, M.D.

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

1. To improve staging procedures.
2. To assess value of "prophylactic" splenectomy.
3. To determine if localized disease is curable
4. To evaluate various therapeutic methods for generalized disease.
5. To assess cross resistance between the drug regimens employed.

Methods Employed:

Staging to include laparotomy and splenectomy in patients not definitely staged as IV by less invasive procedures. Randomization of patients with stage IV disease to prophylactic splenectomy or not. Randomization of stage I and II patients to radiation with or without subsequent chemotherapy. Randomization of stage III and IV patients to MOPP (HN_2, 6 mg./M^2 IV day 1 & 8, VCR 2 mg. IV day 1 & 8, pred. 40 mg./M^2 qd & 14, and procarbazine 100 mgs. p.o. qd X 14) or COP (CTX, 1500 mg./M^2 day 1, VCR 2 mg. day 1 & 8, pred. 40 mg./M^2 qd X 14). These schedules have been changed somewhat from the originals to make them more comparable with each other and with standard VCR, pred. therapy.

Major Findings:

Laparotomy has been of value in increasing diagnostic accuracy but is not 100% accurate. Although both regimens are successful in inducing partial remissions in 92%, the complete response rates for COP (36%) and for MOPP (42%) do not warrant the toxicity encountered. MOPP was generally more toxic than COP with cumulative hematologic toxicity the major drawback. There were 2 drug-related deaths from MOPP, 1 from COP. Of interest is the high rate of response on crossover, 75% on COP (including 2 CR's) and 67% on MOPP.

Significance to Cancer Research:

To enhance our knowledge of the nature and extent of untreated lymphomas; to define the therapeutic role of prophylactic splenectomy and to further evaluate 2 combinations of chemotherapeutic agents, one of which has not been thoroughly investigated in this dosage schedule.

Proposed Course:

Continue the study in patients with apparently localized lymphocytic lymphomas. Otherwise, terminate the study.

Honors and Awards: None

Publications: None

Presentations: Robert S. Benjamin, M.D.
"COP vs MOPP in Non-Hodgkin's Lymphoma". Presented at the Fourth Conjoint Meeting on the Status of Clinical Studies of the NCI, Annapolis, Maryland. January 11, 1973.

Serial No. NCI 6264
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical and Autopsy Observations of Infectious Diseases in Cancer Patients

Previous Serial Number: 6264

Principal Investigator: William H. Greene, M.D.

Other Investigators: Stephen C. Schimpff, M.D., Edward Sickles, M.D., Nancy Cusack, Peter H. Wiernik, M.D.

Cooperating Units: Research Microbiology, Viola M. Young, Ph.D., I.J. Abramson, Ph.D.

Man Years

Total:	7/16
Professional:	5/16
Other:	2/16

Project Description:

Objectives:

Since the advent of platelet concentrates for the treatment of thrombocytopenia, chemotherapy-induced granulocytopenia with attendant infection has become the primary complication of aggressive drug therapy for advanced malignancy. Moreover, even without such therapy, certain forms of malignancy predispose greatly to infection. This project is the logical accompaniment of the one described under serial 6217 for, where the latter is an attempt at prophylaxis, the primary objectives of this investigation focus on the delineation of those factors that have led to clinical infection. Thus, the aims of this study are to 1) catalogue and identify the infections manifest a) premortem, during the clinical course, and b) postmortem, at autopsy; 2) identify the predisposing factors which have contributed to infection within a given patient; c) suggest, by assessment of the manifestations of infection, further diagnostic and/or therapeutic maneuvers considered worthy of pursuit, or previous such measures no longer justified by efficacy.

Methods:

Each patient with a known or suspected infection is seen by one of the investigators who examines the patient and reviews the chart, both initially and

Methods (cont'd.):

throughout the course of the infection. Detailed records of history, physical findings, nursing notes, laboratory data, and microbiologic isolations are kept. A detailed summary of the first two years' observations has been prepared.

In addition, a two-year review of all BCRC patients autopsied during the interval 7/1/69 to 6/30/71 was conducted by surveying the causes of death, infections present at autopsy, etiologic agents and predisposing factors (e.g., granulocytopenia, tumor obstruction, etc.) in the three weeks prior to death.

Major Findings:

A) Overall Clinical Infections - During the 24-month period, 7/1/70-6/30/72, there were 458 infections among the approximately 600 patients seen of which 338 were documented as to etiology. By far, patients with acute nonlymphocytic leukemia had the greatest incidence of infection, accounting for 179 of the total infections and 123 of the documented ones. Other tumor types were considerably less often complicated by infections. The primary identified pathogens over the two-year span were Pseudomonas aeruginosa (on 27 occasions), Staphylococcus aureus (23), E. coli (20), Candida albicans (9), Klebsiella sp. (7). Among the documented infections, the most common sites were the skin with 82 infections, the bronchopulmonary tree which accounted for 73 episodes, the urinary tract with 62, and the anorectum with 19. One hundred and four of the infections were accompanied by bacteremia, of which 27 involved Pseudomonas aeruginosa.

The necessity to analyze the site, organisms and predisposing factors of infection is a reflection of the continuing search for more efficacious antibacterial and antifungal therapy, and more rewarding methods of diagnosis. Thus, the continuing pre-eminence of Pseudomonas aeruginosa as a source of morbidity and mortality obligates the continuation of the use of carbenicillin and gentamicin as empiric therapy (see 6266). Moreover, the growing role of E. coli and Klebsiella in fatal infections suggested the addition of cephalothin. In addition, since pneumonia is the primary site of infection, new diagnostic aids have been invoked, including the use of transtracheal aspiration of sputum and transtracheal bronchial brushing to allow culture material to be obtained from the peripheral lesion itself in those instances in which sputum production is absent.

B) Autopsy-Documented Fungal Infection - Although review of clinically obvious infection provides extensive data for bacterial etiologies, non-bacterial infections (NBI) are usually undiagnosed prior to death. Thus, a review of 120 autopsied BCRC patients was compared with 48 solid tumor Public Health Service Hospital (PHSH) patients and 54 PHSH patients without malignancy to ascertain the incidence of NBI, and to compare the presence of selected factors in the three weeks prior to death. The results of this study revealed that non-bacterial infection was far more common

Major Findings (cont'd.):

than previously realized. Overall, 41 (42.5%) of the 120 BCRC patients had 63 NBI, most of which were confined to patients with acute leukemia or Hodgkin's disease, of whom 60% had NBI at autopsy. Moreover, one-half of these infections, or approximately 30% of all patients with acute leukemia or Hodgkin's disease, were felt to have been the sole cause or a major contributory cause of death. Sixty percent of the infections and 73% of the patients were infected with Candida sp., with Aspergillus sp. being found in 13% of the infections and 16% of the patients infected. Moreover, diagnosis premortem was uncommon - in 41 of the 63 NBI neither the site nor the organism causing the infection was known prior to death. Cytomegalovirus, Toxoplasma gondii and Pneumocystis carinii were rarely found. Thus, the survey has demonstrated a) the frequency of non-bacterial infection among the subgroups of patients with Hodgkin's disease and acute leukemia; b) the necessity for major improvement in our diagnostic techniques for NBI; c) the fact that infectious pulmonary infiltrates in patients with acute leukemia and Hodgkin's disease, at least in our patient population, if unresponsive to anti-bacterial antibiotics, should almost always be treated empirically with amphotericin B, and not with pentamidine or sulfadiazine and pyrimethamine.

C) Factors Affecting Acquisition of Nosocomial Organisms in Patients with Acute Leukemia - Previous studies from this Center have demonstrated the widespread acquisition of gram-negative bacilli, especially Pseudomonas aeruginosa, from the hospital environment. However, factors affecting that acquisition have not been fully explored. In a recent survey undertaken in a relatively uniform population of acute leukemia patients, 768 days of antibiotic therapy were associated with 54 acquisitions of gram-negative bacilli, i.e., one organism/14 days. In contrast, one organism was acquired per 19 days off antibiotics. Considering pathogens only (Pseudomonas, E. coli, Enterobacter, Klebsiella, Hemophilus), similar figures were: 1/20 days on and 1/29 days off antibiotic therapy, especially for Klebsiella sp. - 1/7 on and 1/20 days off antibiotics. Also affecting colonization was the duration of antibiotic therapy. Thus, for days 1-4 of antibiotic therapy, only one organism was acquired per 22 such days as compared to 1/9 and 1/8 for days 5-8 and 9-12 of therapy.

While sex did not affect acquisition, patient age did appear significant - thus, for patients < 40 the overall acquisition was 1/22 days on and 1/26 days off antibiotics, compared to 1/10 and 1/13 respectively in patients > 40 years of age. Analysis of the duration of antibiotic administration within stratified age groups revealed that older patients will acquire nosocomial organisms considerably earlier in the course of therapy than younger patients.

The significance of this lies in the necessity to be circumspect in the institution and, particularly, the continuation of anti-bacterial antibiotic therapy especially in the older patient. This has special importance in regard to the empiric antibiotic protocol outlined in 6266.

Major Findings (cont'd.):

D) Candida Endophthalmitis - As noted above, fungal infection is difficult to diagnose premortem. A clue to such infection may occasionally be seen in the patient who develops fungal endophthalmitis, as did a recent patient with acute nonlymphocytic leukemia. The virtually pathognomonic characteristics of this infection plus its susceptibility to therapy with IV amphotericin B makes its recognition of clinical importance.

E) Abandonment of Nonproductive Diagnostic Tests - A recent review of more than 3600 cerebrospinal fluid and bone marrow aspirate samples cultured fro bacteria, fungi and acid-fast bacilli among largely asymptomatic cancer patients revealed virtually no instances in which an infection not previously suspected was diagnosed. This has led to abandonment of such "routine" cultures with an extraordinary savings in time, cost and effort.

Proposed Course: Continue investigation.

Significance to Cancer Research: A definition of the incidence, etiology, and sites of clinical infection, and an evaluation of the diagnostic techniques extant, are necessary precursors to the rational use and planning of anti-bacterial and anti-fungal therapy and the institution of alternate ways by which to make the diagnosis of infection. The successful control of infection, in turn, is capable of significantly improving the response rate to current anti-neoplastic therapy by allowing sufficient survival for delivery of such therapy.

Publications:

I Dr. Greene

1. Greene, W., Wiernik, P.: Candida Endophthalmitis - Successfully Treated in a Patient with Acute Leukemia. Am J Ophthal 74:1100-1104, 1972.

2. Greene, W., Schimpff, S., Vermeulen, G., Young, V.M., Wiernik, P.: The Value of Routine Bone Marrow and Cerebrospinal Fluid Cultures in Patients with Cancer - A Negative Report. Am J Clin Path (in press).

II Dr. Schimpff

1. Schimpff, S., Serpick, A., Stoler, B., Rumack, B., Mellin, H., Joseph, J., Block, J.: Varicella-zoster infection in patients with cancer. Ann Int Med 76:241-254, 1972.

2. Schimpff, S., Wiernik, P., Block, J.B.: Rectal abscesses in cancer patients. Lancet 2:844-847, 1972.

3. Schimpff, S., Greene, W., Young, V.M., Wiernik, P.: Pseudomonas infections in cancer patients. Contemp Surg, Jan, 1973.

Presentations:

I Dr. Greene

1. Non-Bacterial infections at autopsy in patients with cancer. American Federation for Clinical Research. Atlantic City, New Jersey, April, 1972.

2. The diagnostic value of routine cerebrospinal fluid and bone marrow cultures - a negative report. 12th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, New Jersey, September, 1972.

3. Non-Bacterial complications of patients with acute nonlymphocytic leukemia. Inter-Hospital Infectious Disease Conference, Baltimore, Maryland, March, 1973.

II Dr. Schimpff

1. Empiric use of carbenicillin and gentamicin for presumed infection in granulocytopenic cancer patients. Eastern Section of the American Federation for Clinical Research, New York, New York, December, 1970.

2. Epidemic varicella-zoster infection in cancer patients. 52nd Annual Meeting of the American College of PHysicians. Denver, Colorado, March, 1971.

3. Varicella-zoster infections. Infectious Disease Seminar, University of Maryland, Baltimore, Maryland, October, 1971.

4. Pseudomonas infections in cancer patients. Annual Meeting of the American Society of Contemporary Medicine and Surgery. Hollywood, Florida, March, 1972.

5. Infection in patients with cancer. Grand Rounds, Fitzpatrick Mercy Hospital, Chester, Pennsylvania, March, 1972.

6. Prevention and Treatment of Infections in Patients with Acute Leukemia. D.C. General Hospital, Washington, D.C., September, 1972.

7. Pseudomonas aeruginosa infections in cancer patients. Washington Sanitarium and Chronic Disease Hospital, Washington, D.C., September, 1972.

8. Infections in acute leukemia. Georgetown University School of Medicine, Washington, D.C., October, 1972.

9. Herpes zoster and cancer patients. National Institute of Allergy and Infectious Disease, Bethesda, Maryland, October, 1972.

10. Catheters, IV fluids, blood products, and infection in cancer patients, National Naval Medical Center, Washington, D.C., November, 1972.

Presentations (cont'd.):

11. Surveillance cultures, Pseudomonas aeruginosa and rectal abscesses in patients with cancer. National Naval Medical Center, Washington, D.C., January, 1973.

12. Infections in acute leukemia. University of Maryland School of Medicine, Baltimore, Maryland, February, 1973.

13. Pseudomonas and rectal abscesses in acute leukemia. University of Maryland School of Medicine, Baltimore, Maryland, March, 1973.

Serial No. NCI-6266
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Therapy of Infection in Patients with Cancer

Previous Serial Number: 6266, 6267

Principal Investigator: William H. Greene, M.D.

Other Investigators: Stephen C. Schimpff, M.D., Peter H. Wiernik, M.D., Clarence Fortner, M.S.

Cooperating Units: Research Microbiology, Viola M. Young, Ph.D.

Man Years

Total:	3/4
Professional:	3/4
Other:	0

Project Description:

Objectives:

A) Empiric Antibiotic Therapy for Granulocytopenic, Febrile, Cancer Patients- Infection is a frequent and serious complication in the granulocytopenic patient with cancer. Bacteremia commonly occurs and usually leads to rapid demise. This investigation was designed to assess the efficacy of systemic antibiotics, given as initial empiric therapy, for the therapy of presumed sepsis in granulocytopenic, cancer patients.

B) Use of Cytosine Arabinoside in the Therapy of Varicella-Zoster Infections- Because zoster infections at this cancer center are common and because they have a significant degree of morbidity, and because there is preliminary evidence to suggest that cytosine arabinoside will halt the multiplication of varicella-zoster virus, it was decided to use cytosine arabinoside as systemic therapy of patients with early zoster in a controlled, randomized trial to determine whether this therapy would be better than placebo in halting the progression of infection.

C) Initial Studies of a New Antibiotic Tobramycin - Tobramycin, an aminoglycoside similar to gentamicin, has a spectrum of activity similar to gentamicin. However, in vitro studies suggest that it is two to four times more active than gentamicin against Pseudomonas aeruginosa. Pseudomonas aeruginosa is the most common cause of significant and serious infection at the BCRC. It was, therefore, decided that Tobramycin should be intensively studied at this Center.

Major Findings:

A) Empiric Antibiotic Therapy - The previous study with carbenicillin (C) and gentamicin (G) demonstrated effective treatment of patients with Staphylococcus aureus and Pseudomonas aeruginosa infection, but lesser success among patients infected with Escherichia coli and Klebsiella sp. Thus, cephalothin, to which most E. coli and Klebsiella were found to be susceptible by in vitro testing, was added as part of the empiric therapy for febrile, granulocytopenic patients.

A review of 18 months' experience from 2/71 to 7/72 involving 177 trials in 89 patients revealed that the overall improvement (cure) rate among 94 documented or probable infections was 60%, with temporary improvement in 10% and failure in 30%. The most common pathogen was Pseudomonas aeruginosa which was implicated as causative of infection 24 times, of which 21 were bacteremic. Eleven (45.8%) improved and 10 (41.7%) failed. E. coli and Klebsiella sp. each caused 16 infections of which 68.7% and 43.5% respectively were cured. Fifteen of 16 infections with Staphylococcus aureus or group D streptococcus also responded to therapy.

The most frequent site of origin (34 times) was the bronchopulmonary tree of which only 38.2% improved and 50% failed. In contrast cure was seen in 52.9% of 17 anorectal infections, 88.2% of 17 oro- or naso-pharyngeal infections and 87.5% of 8 urinary tract infections, all of which were complicated by a high incidence of bacteremia.

Age, sex, underlying malignant diagnosis, granulocyte count at onset of infection were all found to be unrelated to eventual outcome of therapy. However, there was an extraordinary and highly significant relationship between a) bacteremia and b) the ability to mobilize granulocytes and response to antibiotics. Patients with bacteremia fared considerably worse than those without (45.1% vs. 76.7% improvement respectively); and patients unable to raise their granulocyte count between the onset and end of therapy were uniformly worse off than those who could. Thus, 75% of those patients able to elevate their granulocyte count were cured compared to 46% unable to do so. Moreover, 10 of 15 bronchopulmonary infections improved with rising granulocyte levels as against only 3 of 19 with stable or falling counts. Similarly, for Pseudomonas infections the comparable figures were 75% and 16.7%, and for Klebsiella infections 85.7% vs. 11.1%.

The major toxicities were renal compromise and superinfection. The former was tolerable, with only 6% of all trials complicated by rises in serum creatinine from initially normal levels to > 3 mg%. Superinfection, often severe, occurred in one-quarter of all trials and was directly related to the duration of broad-spectrum therapy, achieving levels of > 50% in patients treated for 12 days or more.

In comparison to previous experience with cancer patients, the regimen represents an effective method of dealing with bacterial infection. While modifications in the protocol may be made with regard to the duration of therapy for

given infections, or the use of empiric antifungal therapy for unresponsive infections, the basic protocol is expected to continue.

B) Cytosine Arabinoside for Herpes Zoster - Thus far the randomized, controlled double-blind study has had 9 patients entered. It is still too early for significant evaluation, although there has, thus far, been no striking evidence of efficacy for cytosine arabinoside when compared to placebo in our current dosage and route of administration.

C) Tobramycin - The use of Tobramycin in vitro for disc-susceptibility testing of resistant strains of Pseudomonas aeruginosa is now routine at Hazelton Laboratories. The in vivo use of Tobramycin has been confined to only a few patients, in whom no significant advantage over gentamicin could be discerned.

Proposed Course: Continue all studies.

Significance to Cancer Research:

The empiric use of carbenicillin, gentamicin and cephalothin for presumed bacterial sepsis has significantly reduced the morbidity and mortality of Pseudomonas aeruginosa and E. coli sepsis and as such, has permitted prolongation of life so that more adequate cancer chemotherapy could be used. In addition, systematic evaluation of factors predisposing to failure has led to additional suggestions concerning diagnosis, adjunctive therapy, and prognosis.

Honors and Awards: None

Presentations:

I Dr. Greene

1. Empiric carbenicillin, gentamicin and cephalothin therapy for presumed infection in patients with granulocytopenia and cancer. 54th Annual Session of the American College of Physicians, Chicago, Illinois, April, 1973.

II Dr. Schimpff

1. Empiric therapy with carbenicillin and gentamicin for febrile, granulocytopenic cancer paients. Eastern Section of the American Federation for Clinical Research, New York, New York, December 1970.

2. Carbenicillin-gentamicin therapy of cancer patients. Grand Rounds, Veterans Administration Hospital, Baltimore, Maryland, March 1971.

3. Use of carbenicillin in cancer patients. Film presentation made to representatives of Roerig Division of Pfizer Corporation, December 1971.

Presentations (cont'd):

III Mr. Fortner

1. Hyperalimentation as a Predisposing Factor to Bacteremia in Patients with Cancer. Joint Meeting of Clinical Society and Commissioned Officers' Association, Phoenix, Arizona, May 1973.

Publications:

Schimpff, S., Satterlee, W., Young, V.M., and Serpick, A.: Empiric therapy with carbenicillin and gentamicin for febrile, granulocytopenic cancer patients. New Eng J Med 284:1061-1065, 1971.

Serial No.: NCI-6273
1. BCRC
2. Section Medical Oncology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Bacterial Contamination in Platelet Products Stored at 25°C.

Previous Serial Number: Same

Principal Investigator: Donald H. Buchholz, M.D.

Other Investigators: Viola Mae Young, Ph.D., Charles A. Schiffer, M.D., Peter H. Wiernik, M.D.

Cooperating Units: Central Blood Supply; Baltimore Red Cross

Man Years:

Total:	2/3
Professional:	1/6
Other:	3/6

Project Description:

Objectives:

Since 1969 when Murphy and Gardner first described the apparently beneficial effect of room temperature as oppossed to 4°C storage of platelet rich plasma and platelet concentrate, many centers have been keeping platelet products at ambient temperatures for up to 48 to 72 hours after procurement.

Previous investigation at the Baltimore Cancer Research Center has shown that about 1.6% of more than 3,000 individual units of platelet concentrate contained bacteria. These bacteria may proliferate under conditions of ambient temperature storage to numbers sufficient to cause clinical septicemia in recipients. There have been six documented episodes of recipient sepsis directly related to infusion of room temperature stored platelets to leukopenic cancer patients, two of which proved fatal.

In an effort to prevent the growth of bacteria during storage and to evaluate the clinical effectiveness of stored platelets a comparative study of 4°C and 25°C storage was begun.

Methods Employed:

Platelets are obtained from either commercial or volunteer sources and stored as individual units at 25°C or 4°C for 24 to 48 hours. A laminar air flow hood is used at the time of pooling to diminish the possibility of exogenous contamination. Each pool, whether stored at ambient or refrigerator temperature is cultured in brain heart infusion, tryptic soy broth and thiol, at 25°C and 37°C. Cultures are kept at least 21 days and all specimens are subcultured and gram-stained to determine if bacteria are present. Recipient response as determined by post-infusion platelet count increments and post-transfusion cell half life are measured as well as recipient bleeding time by the Ivy bleeding time technique as modified by Mielke. A limited number of recipients receive platelets tagged with Cr^{51} to additionally determine the rate of disappearance of transfused cells. Clinical correlation with respect to cessation of bleeding is also determined.

Major Findings

No major findings have been made at the present time inasmuch as the study has recently been initiated.

Honors and Awards: None

Presentations, Donald H. Buchholz:

1. Supportive Care for Patients with Malignancy. NCI Brain Tumor Study Group, Cockeysville, Maryland, September, 1972.

2. Current Methods of Supportive Care using Specific Blood Components. Johns Hopkins School of Medicine, Baltimore, Maryland, October, 1972.

3. The Platelet and its Role in Hemostasis. University of Maryland School of Medicine, Baltimore, Maryland, November, 1972.

4. Current Concepts of Blood Component Therapy. St. Barnabas Medical Center, Livingstone, New Jersey, April, 1973.

5. Blood Component Therapy: Cell Supportive Therapy. Baltimore Blood Club, Baltimore, Maryland, May, 1973.

6. The Clinical Use of Granulocytes and Platelets in the Supportive Care of Patients with Malignancy. American Red Cross, Seattle, Washington, May, 1973.

Publications:

D. H. Buchholz, V. M. Young, N. R. Friedman, J. A. Reilly and M. R. Mardiney, Jr. Detection and Quantitation of Bacteria in Platelet Products Stored at Ambient Temperature. Transfusion, in press.

D. H. Buchholz. Blood Transfusion: Merits of Component Therapy. I. The Clinical Use of Red Cells, Granulocytes and Platelets. Journal of Pediatrics, in press. (by invitation).

Serial No.:NCI-6275
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cell Transfusion Therapy

Previous Serial Number: Same

Principal Investigator: D.H. Buchholz, M.D.

Other Investigators: Charles A. Schiffer, M.D.
Peter H. Wiernik, M.D.

Cooperating Units: Baltimore Red Cross; Central Blood Service

Man Years:

Total:	3 1/2
Professional:	1 1/4
Other:	2 1/4

Project Description:

Objectives:

With the advent of newer aggressive chemotherapeutic approaches to the treatment of malignancy, problems arising from secondary suppression of the bone marrow have become more prominent and have led to increased risk of infection and/or hemorrhage. Replacement therapy should substantially decrease these patient risks.

Methods Employed:

I. Platelet Transfusion

Platelets are purchased from the Baltimore Red Cross and from the Central Blood Service. Additionally plateletpheresis is performed on site using HL-A compatible family members as well as HL-A compatible unrelated donors. Platelets are also obtained from patients with acute leukemia in remission and frozen for future use using 5% dimethylsulfoxide and liquid nitrogen storage temperatures. Random donor platelets are prepared and stored at ambient temperature for up to 48 hours although most are transfused fresh. Pre-transfusion and 1-,3-, and 14 hour post-infusion recipient specimens are obtained to evaluate patient transfusion response. A patient-oriented weekly computer summary of information relating to cell transfusion and recipient response was developed to aid in evaluation of the cell trans-

I. Platelet Transfusion (Cont'd)
fusion program.

II. Granulocyte Transfusion

Granulocytes are obtained from normal donors by continuous flow reversible leukoadhesion using nylon fiber filters (Leuko-Pak) and a small peristaltic pump. Clinical response to granulocyte transfusion is determined as is the in vitro function of the collected cells. Donors are closely monitored to ascertain the effectiveness and safety of repeated donation by this technique.

Major Findings:

I. Platelet Transfusion

Platelets prepared and stored at ambient temperature were used for patient transfusion. A significant risk of bacterial proliferation in such stored platelets was demonstrated. Recipients refractory to random donor transfusion have received platelets from HL-A compatible family members with excellent response. A limited number of unrelated HL-A compatible or identical donors have also been used; not all provided anticipated good responses in the transfused recipient. The use of autologous platelets obtained from patients with leukemia in remission have significantly decreased the need for transfusion of random donor cells at times of further chemotherapy; several patients have received only their own cells during relapse with good to excellent result. Techniques to improve platelet freezing are still under development and can not be evaluated at the present time.

II. Granulocyte Transfusion

Granulocyte transfusions have been administered prophylactically as well as for treatment of suspected or documented septicemia or other bacterial infections. Cell yield with the nylon fiber filtration system was proportional to the volume of blood processed and the absolute granulocyte count of the donor. Repeated donations were possible over a short period of time and the donation procedure appears safe for the donor. Cell yield averaged 39.8×10^9 granulocytes per three hour donation when a two filter system was used. In cooperation with Fenwal Laboratories, a sterile closed system with filters in-line was developed for use with a small and inexpensive peristaltic pump. The use of such a system allows significantly greater numbers of granulocytes (six to eight fold) to be harvested from normal non-stimulated donors than is possible with the expensive NCI-IBM Blood Cell Separator. Collected cells are greater than 95% viable and are capable of in vitro phagocytosis and bactericidal activity as well as in vivo circulation and migration into tissues as demonstrated by the Rebuck window technique. An evaluation of the clinical effectiveness of these cells in preventing and combating infection in leukopenic cancer patients is currently underway.

Proposed Course:

I. Platelet Transfusion

Platelet transfusions will continue to be given to patients who become thrombocytopenic following chemotherapy. The plateletpheresis program utilizing HL-A compatible related or unrelated donors will be expanded for more effective treatment of patients refractory to random donor platelet infusions. The importance of freezing rate in relationship to cryoprotective agents used for platelet freezing will be evaluated to provide better post-freezing cellular viability.

II. Granulocyte Transfusion

The use of granulocytes collected by reversible leukoadhesion will continue to be evaluated by clinical observation of patients and responses to infection as well as by in vitro techniques to determine cell function.

Honors and Awards: None

Presentations: D.H. Buchholz, M.D.

1. The Collection of Normal Granulocytes by Means of Reversible Leukoadhesion. University of Miami School of Medicine, Miami, Florida, July, 1972.
2. D.H. Buchholz, P.H. Wiernik and M.R. Mardiney, Jr. Granulocyte Procurement by Leukocyte Filtration. Presented at the XXV Annual Meeting of the American Association of Blood Banks-XIII Transfusion Congress, International Society for Blood Transfusion, Washington, D.C., August, 1972.
3. D.H. Buchholz, L.A. Yamamoto and J.K. Hooper. Hematologic Parameters and Transfusion: Weekly Summary by Computer. Presented at the XXV Annual Meeting of the American Association of Blood Banks-XIII Transfusion Congress, International Society for Blood Transfusion, Washington, D.C.,August, 1972.
4. Current and Investigational Methods of Granulocyte Harvest for Transfusion. Johns Hopkins School of Medicine, Baltimore, Maryland, September, 1972.
5. Supportive Transfusion Care of Patients with Malignancy. NCI Brain Tumor Study Group, Cockeysville, Maryland, September, 1972.
6. Current Methods of Supportive Care using Specific Blood Components. Johns Hopkins School of Medicine, Baltimore, Maryland, October, 1972.
7. The Platelet and its Role in Hemostasis. University of Maryland School of Medicine, Baltimore, Maryland, November, 1972.
8. Granulocyte Collection by Continuous Flow Reversible Leukoadhesion. Annual Meeting, Fenwal Laboratories, Tarpon Springs, Florida, March, 1973.
9. Current Concepts of Blood Component Therapy. St. Barnabas Medical Center Livingstone, N.J., April, 1973.
10. Blood Component Therapy: Cell Supportive Therapy. Baltimore Blood Club, Baltimore, Maryland, May, 1973.
11. Granulocyte Harvest for Transfusion. American Red Cross, Portland, Oregon, May, 1973.
12. The Clinical Use of Granulocytes and Platelets in the Supportive Care of Patients with Malignancy, American Red Cross, Seattle, Washington,May,1973.

Publications:

1. D.H. Buchholz, V.M. Young, N.R. Friedman, J.A. Reilly and M.R. Mardiney, Jr. Bacterial Proliferation in Platelet Products Stored at Room Temperature. New England Journal of Medicine 285:429, 1971.
2. D.H. Buchholz, V.M. Young, N.R. Friedman, J.A. Reilly and M.R. Mardiney, Jr. Detection and Quantitation of Bacteria in Platelet Products Stored at Ambient Temperature. Transfusion, in press.
3. D.H. Buchholz. Blood Transfusion: Merits of Component Therapy. I. The Clinical Use of Red Cells, Platelets and Granulocytes. Journal of Pediatrics, in press (by invitation).
4. D.H. Buchholz. Blood Transfusion: Merits of Component Therapy. II. The Clinical Use of Plasma and Plasma Components. Journal of Pediatrics, in press (by invitation).

Serial No.:NCI-6276
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Chemotherapy of Metastatic Testicular Carcinoma

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert Esterhay, Jr., M.D.
Michael O'Connell, M.D.
Edward Sickles, M.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives:

To access the efficacy of various anti-tumor chemotherapeutic agents in metastatic testicular carcinoma.

Methods Employed:

Patients are currently randomized between a combination of drugs consisting of fluorouracil, cyclophosphamide, methotrexate, and vincristine and a combination including the same drugs plus bleomycin given 12 hours after the dose of vincristine. Patients failing this protocol are randomized between adriamycin or IV methyl CCNU.

Major Findings:

Of 15 patients entered into this study, 7 on one arm and 8 on the other, 12 have shown substantial clinically useful response. The numbers are still too small to determine whether or not one arm of this treatment protocol is superior to the other, but early data suggests that the 5 drug combination may be superior. Toxicity has been minimal.

Significance to Cancer Research:

Testicular carcinomas account for a significant proportion of tumors in young male adults. Metastatic testicular carcinoma has been shown to be responsive to several different classes of anti-tumor agents by this group and others. The present study shows promise of achieving results superior to those previously reported.

Proposed Course:

Continue present studies.

Honors and Awards:None

Publications:

1. O'Connell, M.J. and Wiernik, P.H.: Remissions of Metastatic Testicular Cancer. N. Engl. J. Med. 287:989, 1972 (letter to the Editor).
2. Belliveau, R.E., Wiernik, P.H., Sickles, E.A.: Blood Carcinoembryonic Antigen, Regan Isoenzyme, and Human Chorionic Gonadotropin in a Man with Primary Mediastinal Choriocarcinoma. Lancet 1:22-23, 1973.
3. Sickles, E.A., Sklansky, B.D. and Wiernik, P.H.:Benign Intrathoracic Lesions Mimicking Recurrent Cancer. JAMA, in press.

Serial No.:NCI-6277
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Chemotherapy of Adult Acute Leukemia

Previous Serial Number: None

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: William H. Greene, M.D.
Michael J. O'Connell, M.D.
Charles A. Schiffer, M.D.
Robert S. Benjamin, M.D.
Nicholas R. Bachur, M.D.
Richard Kaplan, M.D.
Sol Spiegelman, M.D.
Dwight Stickney, M.D.

Cooperating Units: Acute Leukemia Cooperative Group B
Research Microbiology, BCRC
Patient Care Pharmacy, BCRC
Section of Biochemistry, Laboratory of Pharmacology, BCRC
Section of Neurological Surgery, BCRC
Francis Delafield Hospital, New York
Burroughs-Wellcome Company, North Carolina

Man Years:

Total:	6
Professional:	3
Other:	3

Project Description:

Objectives:

1) To evaluate the effectiveness of newer chemotherapeutic agents alone and in combination against adult acute leukemia.
2) To correlate clinical results with pharmacokinetic data.
3) To define the usefulness of newer chemotherapeutic agents in Phase II trials in adult acute leukemia.
4) To identify factors of prognostic import in adult acute non-lymphocytic leukemia.
5) To study the effect of intensive anti-leukemic therapy during complete hematologic remission on the duration of remission.

Objectives: (Cont'd)

6) To identify biochemical indicators of occult disease activity in adult patients with acute non-lymphocytic and lymphocytic leukemia.

Methods Employed:

Protocol studies of various anti-tumor agents alone or in combination have been initiated. In addition muramidase has been measured by several methods, carcinoembryonic antigen by radioimmunoassay and immunoglobulin concentration in serum and other body fluids by immunoelectrophoresis.

Major Findings:

I. Effect of Single Agents

A) Daunorubicin:
Daunorubicin has been found in previous studies at this institution to be the most effective single agent in the treatment of adults with acute non-lymphocytic leukemia. Consequently daunorubicin given in the dosage and schedule identified by our pharmokinetic studies with the Section of Biochemistry as the most efficacious was utilized as one arm of a controlled Phase III study in adult acute non-lymphocytic leukemia. To date 35 patients have been entered on-study. 23 are evaluable at the time of this report. The complete remission rate with this drug alone is 48% of all patients entered on protocol. If patients who died within 10 days of hospitalization are omitted from evaluation, the complete remission rate is 59%. If all patients over the age of 70 are omitted from evaluation the complete remission rate is 69%. The median survival of patients treated with daunorubicin alone as of the date of this report is 158+ days with a range of 5-488+ days. These calculations are made at a time when 6 of the 23 patients are still alive and 2 of those 6 are alive and below the median. The median survival of all patients who achieved a complete response is 292+ days with 8 of the 11 complete responders still alive and 3 of those 8 still alive below the median. These data confirm and extend our previous observations with daunorubicin.

B) Azacytidine vs. Guanazole:
This is a Phase II study designed to determine the relative efficacy of two agents recently demonstrated to have activity in acute leukemia, namely azacytidine and guanazole. 3 patients have been entered on this study to date and it is too early to evaluate it for results.

II. Effect of Multiple Drugs in Combination

A) Daunorubicin, Cytosine Arabinoside, Thioguanine and Pyrimethamine:
This program forms the second randomization in our current protocol for adult acute non-lymphocytic leukemia. Pyrimethamine is included in this combination of agents in an attempt to treat occult central nervous

A) Daunorubicin, Cytosine Arabinoside, Thioguanine and Pyrimethamine: (Cont'd)
system leukemia at the time of diagnosis with an agent that can be given orally. Researchers at Burroughs-Wellcome and Company, Research Triangle, North Carolina have measured pyrimethamine levels in cerebral spinal fluid from our patients treated on this arm of the study and have identified the compound in adequate concentration for prolonged periods of time. Thirty-six patients have been entered on this arm of the protocol, 22 of which were evaluable at the time of this report. The complete remission rate for all entered patients is 52%. If patients who died within 10 days of admission are excluded from the evaluation the complete remission rate is 55%. If all patients over the age of 70 are excluded from evaluation the complete remission rate is 65%. The median survival of patients on this arm of the study is 120+ days with 5 of the 22 evaluable patients still alive and 1 of those 5 alive below the median. The median survival of patients who achieved a complete response is 268+ days with 5 of the 11 complete responders still alive and 3 of those 5 alive below the median. There is evidence from this study, although not yet statistically significant, that the administration of pyrimethamine at the time of initial therapy in adult patients with acute non-lymphocytic leukemia is useful in treating occult meningeal leukemia.

B) Daunorubicin, Cytosine Arabinoside and Thioguanine; Cytosine Arabinoside and Thioguanine; and Daunorubicin Alone:
This study is being chaired by the principal investigator for Acute Leukemia Group B (ALB# 7221). As of the time of this report 110 patients have been entered and 57 are evaluable. The complete remission rate is over 50% in each arm of the study, but it is too early to evaluate duration of remission. The study will continue during the current fiscal year.

C) Vincristine, Thioguanine, Pyrimethamine and Dexamethasone vs. Vincristine Thioguanine, Methotrexate and Prednisone for Remission Induction in Adults with Acute Lymphocytic Leukemia:
This program is designed to test whether or not pyrimethamine administered initially to adults with acute lymphocytic leukemia can prevent or delay the onset of meningeal leukemia in long-term survivors. Additionally the protocol was designed to test whether or not dexamethasone, a steroid that mobilizes granulocytes might be associated with fewer infections during induction than prednisone, a steroid that inhibits granulocyte mobilization. To date, 8 patients have been entered and it is too early to evaluate the results.

III. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

A) Cytoxan and Guanazole:
All patients achieving complete remission on the daunorubicin alone arm or the daunorubicin, cytosine arabinoside, thioguanine and pyrimethamine arm of our protocol #7110 are maintained with monthly injections

A) Cytoxan and Guanazole: (Cont'd)
of cytoxan 1500 mg per sq. meter of body surface area followed in 18 hours by guanazole 25 grams per sq. meter in a 24 hour IV infusion. It is hoped that cells in G_0 phase might be recruited into the cell cycle by the agent cytoxan. Subsequent treatment with guanazole, an S-phase active agent, might then accomplish a greater degree of cell kill than if used alone. The combination of these agents used in this way might thereby prolong the duration of remission over that which is customarily achieved in this disease. At present the median duration of first remission in both arms of the study is approximately 6 months with many patients still in remission below the median. This figure compares favorably with the 2.3 months median duration of unmaintained remission seen in a previous study here utilizing daunorubicin or POMP

IV. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia.

A) Serum and Urine Muramidase Studies:
Our studies in serum and urine muramidase activity in adults with acute non-lymphocytic leukemia continue. We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B) Carcinoembryonic Antigen:
We have observed that approximately 30% of adult acute non-lymphocytic leukemia and 30% of adults with acute lymphocytic leukemia have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse the presence or absence of carcinoembryonic antigen in their plasma at that time does not correlate with findings at the time of presentation

C) Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia:
We have measured quanitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved these levels become normal. Investigation continues into the question whether or not serial immunoglobulin levels might be useful in determining the disease status in a patient with acute non-lymphocytic leukemia.

D) Reverse Transcriptase in Leukemic Cells:
This enzyme of presumed viral origin is being looked for in cells and plasma from our leukemia patients by Dr. Sol Spiegelman, New York. Results are preliminary at this point.

Summary of Major Findings:

1. Daunorubicin alone may be as effective an inducer agent for adult acute non-lymphocytic leukemia as the other combinations of agents tested. Since remissions are generally achieved more quickly with daunorubicin alone than with combinations of agents, daunorubicin may ultimately prove to be superior for remission induction when compared to other presently used combinations of agents.
2. Pyrimethamine may be useful in meningeal leukemia prophylaxis in adults with acute non-lymphocytic leukemia.
3. Serum muramidase levels are useful in following disease activity in adult acute non-lymphocytic leukemia.
4. Carcinoembryonic antigen is present in increased amounts in the minority of patients with acute non-lymphocytic leukemia. However serial determinations are useless in following disease activity.

Significance to Cancer Research:

These studies indicate continued improvement in our ability to arrest disease activity in adults with acute non-lymphocytic leukemia in an ever increasing number of patients. In addition biochemical parameters are being identified that reflect accurately disease activity in many patients with acute non-lymphocytic leukemia. The identification of such factors may lead to more rational therapy in the future. Demonstration of reverse transcriptase in leukemic cells may have etiologic significance.

Proposed Course of Project:

These investigations will be continued through the fiscal year.

Honors and Awards: None.

Invited Lectures: Peter H. Wiernik, M.D.

1) University of Maryland School of Medicine - December, 1972
2) Visiting Professor; Royal Victoria Hospital, McGill University School of Medicine, Montreal,P.G. Canada - December, 1972
3) St. Joseph's Hospital, Baltimore, Maryland - January, 1973
4) Western Reserve University School of Medicine - February, 1973
5) Greater Baltimore Medical Center - February, 1973
6) Johns Hopkins University School of Medicine - March, 1973
7) Grand Rounds at National Cancer Institute, Bethesda - March, 1973
8) Bon Secours Hospital, Baltimore, Maryland - April, 1973

Publications:

1. Wiernik, P.H.: Treatment of Acute Granulocytic Leukemia in Adults, N. Engl. J. Med. 287:308, 1972.
2. Rosenoff, S.H., Canellos, G.P., O'Connell, M. and Wiernik, P.H.: Mediastinal Adenopathy in Granulocytic Leukemia, Arch. Int. Med.1973(in press).
3. Greene, W., Schimpff, S. and Wiernik, P.: Cell Mediated Immunity and Prognosis in Acute Non-Lymphocytic Leukemia. Proc. Amer. Soc. Hemat. 1972, p.40

Serial No.: NCI-6278
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Determination of Serum Alpha Fetoprotein and Plasma Carcinoembryonic Antigen in Patients with Malignant Disease.

Previous Serial Number: NCI-6278

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert J. Esterhay, Jr., M.D.
K.R. McIntire, M.D.

Cooperating Units: Laboratory of Cell Biology, NCI
Hoffman-La Roche, Medical Research Department, Nutley, New Jersey
Section of Neurological Surgery, BCRC

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

To determine whether or not carcinoembryonic antigen and alpha fetoprotein are present in the serum or plasma of patients with a variety of malignant diseases. In addition to determine whether or not serial determinations of alpha fetoprotein and carcinoembryonic antigen in patients with initially elevated levels are useful in following disease activity.

Methods Employed:

Carcinoembryonic antigen and alpha fetoprotein are determined by radioimmunoassay. Patients are investigated prior to any therapy and those with positive results are followed serially throughout their course.

Major Findings:

1. Carcinoembryonic antigen is present in the plasma of patients with a variety of neoplasms including hematologic malignancies, brain tumors, neuroblastoma, gastrointestinal tumors and others.
2. In some patients plasma carcinoembryonic antigen levels accurately reflect disease activity.
3. Alpha fetoprotein elevations in sera are not specific for patients with hepatocellular carcinoma. Elevated alpha fetoprotein is found in about 25% of patients with embryonal cell carcinoma of the testes and teratocarcinoma of the ovary. In addition a rare patient with gastric carcinoma with elevated serum alpha fetoprotein can be identified.

Significance to Cancer Research:

The identification of biochemical parameters that may signal the presence of occult tumors is of prime importance to optimal therapy of cancer.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications:

Esterhay, R.J., Jr., Shapiro, H.M., Sutherland, J.C., McIntire, K.R. and Wiernik, P.H.: Serum Alpha Fetoprotein Concentration and Tumor Growth Dissociation in a Patient with Ovarian Teratocarcinoma. Cancer, in press, 1973.

Presentations:

Wiernik, P.H. and Serpick, A.A.: Plasma Carcinoembryonic Antigen (CEA) in Patients with Advanced Cancer. American Association of Cancer Research, Atlantic City, New Jersey, April, 1973.

Serial No.: NCI-6279
1. BCRC
2. Biochemistry Section and Medical Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Initial Clinical and Pharmacological Evaluation of Adriamycin

Previous Serial Number: NCI-6279

Principal Investigator: Robert Benjamin, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Nicholas Bachur, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives:

1. To determine the effectiveness and toxicity of adriamycin given by a single intermittent high-dosage schedule in a variety of malignancies.
2. To study the pharmacokintics of adriamycin in these patients.
3. To make clinical and pharmacologic correlations.

Methods:

Any patient with an unresectable neoplasm not specifically encompassed by preexisting BCRC protocols is eligible for the study. Particular emphasis is to be placed on patients with sarcomas. Standard dosage is 60 mg/M^2 repeated in 3-4 weeks after resolution of toxicity from the previous dose. In patients with severe liver disease (BSP>15% and/or bilirubin>3 mg%) dosage is reduced by 75%. In patients with moderate liver disease (BSP 9-15%, bilirubin 1.2-3 mg%) dosage is reduced by 50%. Serial samples of plasma and urine will be collected on selected patients and analyzed by spectrophotofluorometry and thin-layer chromatography for drug and metabolites.

Major Findings:

Clinical Efficacy

Eighty-two of 96 patients are evaluable. Responses have been seen in a variety of tumors. In 26 patients with hematologic neoplasms, 4 obtained complete remission (2-nodular PDL lymphomas, 1 diffuse histiocytic lymphoma, 1 ALL); 11 obtained partial remission, and 6 improved. The remission rate in pure and mixed histiocytic lymphomas (CR + PR = 5/7 = 86%) and lymphocytic lymphomas (CR + PR = 5/8 = 62%) is particularly noteworthy. In sarcomas, complete remissions have been seen in patients with myxofibrosarcoma and soft-tissue Ewing's sarcoma. Partial remissions were most common in rhabdomyosarcoma (4/5) but were also seen in osteosarcoma, leiomyosarcoma, mesothelioma, and giant-cell tumor of bone. Improvement was noted as well in patients with liposarcoma and malignant fibrous histiocytoma. Overall remission rate (CR + PR) was 10/25 or 40%, response rate was 16/25 or 64%. In carcinomas, best results were seen in tumors of lung, breast, and testis. No complete remissions were seen, but 5/10 with lung cancer (2-adenocarcinoma, 1 large-cell undifferentiated carcinoma, 2 small cell carcinoma) had partial remissions and 2 (1-adenocarcinoma, 1-squamous carcinoma) improved). In breast cancer, 3/5 had partial remissions and the other two improved. In testicular carcinoma, 1/5 had partial remission and 2 others improved. Partial remission was also seen in 1 patient with anaplastic carcinoma of the thyroid, and improvement was seen in patients with neuroblastoma, malignant teratoma of the ovary, and adenocarcinoma of unknown primary site. Overall remission rate for patients with carcinomas was 10/31 = 32%; response rate was 19/31 = 61%. Median duration of remission was 11+ weeks for hematologic malignancies, 18+ for sarcomas and 15+ for carcinomas. Survival in sarcomas was significantly prolonged. Six patients with solid tumors showed CNS progression despite systemic response.

Clinical Toxicity

Major toxicity included bone-marrow suppression, mucositis, and cardiomyopathy. Cardiac toxicity was questionably present in only 1 case (1%). Mucositis was infrequent (9%) due to the intermittent single high-dosage schedule. Hematologic toxicity characterized by leukopenia was present in 86%. Thrombocytopenia (in 3/49) and severe leukopenia (in 1/49) was rare in the absence of 3 risk factors--prior radiotherapy (but not chemotherapy), bone-marrow infiltration, and severe liver disease without dosage reduction. In 8 patients with liver disease but without dosage reduction, toxicity was severe--all had marked pancytopenia, 3/8 (38%) died because of marrow depression, and 3/8 (38%) had severe mucositis.

Pharmacokinetics

Mean long plasma half-live of total drug fluorescence was 26.7 hours; relative volume of distribution was 334 L/M^2. For adriamycin itself the mean long half-life was 16.7 hours; relative volume of distribution was 1780 L/M^2. Cumulative urinary excretion of drug and metabolites was 5.7% of the administered dose of which about 60% was adriamycin. In plasma, levels of adriamycin were low compared with the level of pooled metabolites. In patients with severe liver

disease treated with full dosage, plasma levels of drug and metabolites were 4-5 times normal, and elimination was irregular and was greatly delayed. In patients with liver disease treated with reduced dosage, levels of drug and metabolites approached those in patients without liver disease treated with full dosage. Neither adriamycin nor its metabolites were detectable in CSF.

Clinical-Pharmacologic Correlations

Adriamycin dosage need not be adjusted in the presence of renal insufficiency but 50-75% reductions in dosage are necessary to prevent life-threatening toxicity in patients with severe liver disease. When so treated, these patients show response rates, toxicity, and plasma levels similar to those in patients without liver disease treated with full dosage. Due to limited penetration into the CNS, adriamycin may not be effective in controlling CNS metastes from sensitive tumors. The prolonged plasma levels after a single dose make repeated doses unnecessary and more toxic, especially with regard to mucositis.

Significance to Cancer Research:

The basic pharmacokinetics of adriamycin in man has been elucidated. Utilizing the pharmacologic data as the basis of a clinical dosage schedule, we have devised the intermittent single hihg-dosage regimen described. We have singled out 3 risk factors for the development of severe toxicity and devised a way of eliminating the excess toxicity in patients with liver disease without sacrificing drug efficacy. When adriamycin is given according to our regimen, the therapeutic index is significantly increased, for our results are at least as good as any others reported and our toxicity significantly lower.

Proposed Course:

Discontinue the study and formulate new phase III studies utilizing adriamycin singly or in combination with other agents in specific groups of tumors.

Honors and Awards:

Accepted in final competition for the J. D. Lane Award of the USPHS Professional Associations.

Publications:

Robert S. Benjamin, David H. Huffman, Peter H. Wiernik, and Nicholas R. Bachur: Pharmacokinetics of Adriamycin (A) in Man, Proc. Amer. Assoc. Cancer Res. 13: 115, 1972 (Abstract).

R. S. Benjamin, P. H. Wiernik, D. H. Huffman, and N. R. Bachur: Clinical and Biochemical Studies of Intermittent Adriamycin (A) and Daunorubicin (D) Dosage Schedules in Solid Tumor Pations, Clin. Res. 20:563, 1972 (Abstract).

Robert S. Benjamin, David H. Huffman, Peter H. Wiernik and Nicholas R. Bachur: Pharmacokinetics and Metabolism of Adriamycin (A) in Patients with Hematologic Neoplasms and Other Malignancies, Abstracts XIV Int. Cong. Hematology, 510, 1972. Presented by Dr. Benjamin 7/20/72.

Robert S. Benjamin, Charles E. Riggs, Jr., and Nicholas R. Bachur: The Pharmacokinetics and Metabolism of Adriamycin in Man, Clin. Pharmacol. Therap. In press.

Clinical Experience with Adriamycin presented at the Third Joint Working Conference, DCT, NCI, Hunt Valley Inn, 10/3/72 by Dr. Peter Wiernik.

Treatment of Sarcomas presented at the Fourth Conjoint Meeting on the Status of Clinical Studies of the NCI, Annapolis, 1/11/73 by Dr. Peter Wiernik.

Robert S. Benjamin, Peter H. Wiernik, and Nicholas R. Bachur: Adriamycin (AMN)-Efficacy, Safety, and Pharmacologic Basis of a Single Dosage Schedule, Abstracts, Ninth Annual Scientific Meeting, American Society of Clinical Oncology, 35, 1973, presented by Dr. Robert Benjamin, 4/10/73.

Robert S. Benjamin, Peter H. Wiernik, and Nicholas R. Bachur: Adriamycin: A New Effective Therapy for Metastatic Sarcomas, Abstracts, American College of Physicians Fifty-fourth Annual Session, 70, 1973, presented by Dr. Benjamin, 4/12/73.

Robert S. Benjamin, Peter H. Wiernik, and Nicholas R. Bachur: Adriamycin Chemotherapy: Clinical Pharmacology Correlation, to be presented at the 8th Joint Meeting of the USPHS Professional Associations, May 10, 1973 by Dr. Benjamin.

Serial No.:NCI-6280
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Combination Chemotherapy for Stage III-B and IV Hodgkin's Disease

Previous Serial Number: None

Principal Investigators: Peter H. Wiernik, M.D.

Other Investigators: Robert S. Benjamin, M.D.
Michael J. O'Connell, M.D.

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine whether a combination of cyclophosphamide, vinblastine, procarbazine, and prednisone might prove efficacious in the therapy of advanced Hodgkin's disease.

Major Findings:

20 patients have been entered on the study. Prior chemotherapy (usually MOPP) appears to have decreased the response rate whereas prior radiotherapy has not. In patients with prior radiotherapy or no prior therapy the complete response rate of 71% compares favorably with other combinations. It is too early to define the duration of remission.

Significance to Cancer Research:

Although many combinations of effective agents have been identified for the treatment of advanced Hodgkin's disease, in the best hands 20% of patients fail to respond significantly. If this study will result in a regimen that produces a longer duration of response than obtained with other regimens, the therapy of disseminated Hodgkin's disease will have been improved.

Proposed Course:

Continue present study.

Honors and Awards: None

Publications: None

Presentations: Peter H. Wiernik, M.D.
Hematology Division Seminar
Department of Medicine
Johns Hopkins University School of Medicine
December, 1971

Serial No.:NCI-6281
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Initial Clinical Trials of Streptozotocin in the Treatment of Metastatic Cancer

Previous Serial Number: Same

Principal Investigators: Peter H. Wiernik, M.D.
Michael J. O'Connell, M.D.

Other Investigators: None

Cooperative Units: None

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To determine whether or not streptozotocin is a useful therapeutic agent in human neoplastic diseases, to define its spectrum of clinical activity, and to determine the optimal dosage when this agent is administered on a weekly basis.

Methods Employed:

Patients with a wide variety of malignancies were treated with intravenous streptozotocin on a weekly basis with varying dosage schedules as summarized below in an attempt to define the most acceptable dosage regimen.

Major Findings:

This protocol was closed to entry March 1, 1973. There were 36 evaluable patients. There were no complete remissions observed in any tumor category. 1 patient with a malignant carcinoid tumor, 1 patient with a poorly differentiated lymphocytic lymphoma, 1 patient with acute lymphatic leukemia, and 1 patient with a rhabdomyosarcoma achieved partial remission. An additional patient with a poorly differentiated lymphocytic lymphoma as well as 1 patient with malignant melanoma and 1 patient with malignant fibrous histiocytoma

Major Findings: (Cont'd)

experienced improvement as manifested by objective tumor regression less than required for partial remission status. 1 patient with a leiomyosarcoma and 1 patient with a neuroblastoma had stabilization of previously rapidly progressive disease.

Six of the 7 patients who experienced either partial remission or improvement did so with the highest single dose equal to or less than 1.5 grams per meter squared. There was no apparent relationship between disease response and total dose administered.

The major form of drug toxicity was nephrotoxicity. Four evaluable patients had normal pre-treatment BUN and serum creatinine determinations who subsequently developed azotemia on therapy. In each case the patient received at least 1 dose of streptozotocin in excess of 1.5 grams per meter squared. Some evidence of renal tubular dysfunction was observed in approximately 1/2 the patients in which the appropriate studies were performed, regardless of dosage regimen.

In summary, antineoplastic activity against sarcomas, poorly differentiated lymphocytic lymphomas, malignant carcinoid tumor, and acute lymphatic leukemia has been demonstrated using streptozotocin in a weekly dosage regimen of 1.5 grams per meter squared or less. Serious drug-related renal toxicity was observed only in patients receiving doses in excess of 1.5 grams per meter squared.

Significance to Cancer Research:

Streptozotocin is an agent of potential therapeutic value that is not significantly myelosuppressive. The spectrum of clinical activity has been further defined. An optimal dosage level has been arrived at when the drug is used on a weekly dosage regimen. This information is currently being utilized in recently activated protocols for the therapy of malignant carcinoid tumors and insulinomas, lymphoproliferative diseases, and metastatic sarcomas at the Baltimore Cancer Research Center.

Proposed Course:

This protocol was closed to patient entry effective March 1, 1973.

Honors and Awards: None

Publications: None

Serial No.:NCI-6293
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Teaching and Consultation Service in Medical Oncology

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: William Greene, M.D.
Robert Esterhay, Jr., M.D.
Michael O'Connell, M.D.
Charles Schiffer, M.D.

Cooperating Units: University of Maryland School of Medicine
Johns Hopkins University School of Medicine
Greater Baltimore Medical Center
Maryland General Hospital
The Bryman Medix School

Man Years:

Total:	1 1/2
Professional:	1 1/2
Other:	0

Project Description:

Objectives:

To teach the theory and practice of medical oncology to medical students and house officers. To acquaint such individuals with newer aspects of chemotherapy, radiotherapy and surgery as they apply to neoplastic diseases. To provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area free of charge. To teach basics of cancer nursing to physician assistant students.

Methods Employed:

Second year medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition house officers from The Greater Baltimore Medical Center and Maryland General Hospital have been assigned here for elective periods of their house officer

Methods Employed: (Cont'd)

year.

Physicians in the community refer patients here for an out-patient consultation. If the patient meets the qualifications for one of our protocol studies he is accepted in transfer and admitted to the BCRC. If not the patient is completely evaluated as an out-patient and returned to his physician with specific recommendations for therapy. Physician assistant students from the Bryman School are assigned to the wards and out-patient clinic of the BCRC for instruction in the basics of nursing in oncology.

Major Findings:

1. Students, house officers, and physician assistants who have rotated through the BCRC are recognizing that medical oncology is a bonafide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care in order to achieve good results.
2. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

Significance to Cancer Research:

The dissemination of information concerning the latest techniques in diagnosis of therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at an early time, such as during their student years and house officer years such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions which we engage in are of significant import in elevating the level of care available to patients with cancer in this area

Proposed Course:

Continue project.

Honors and Awards: None

Publications: None

Lectures:

1. Peter H. Wiernik, M.D.: Attending Physician, General Medical Ward, Greater Baltimore Medical Center, February, 1972.
2. Peter H. Wiernik, M.D.: Graduation Address. A Need for a Comprehensive Health Care Team, The Bryman School, June, 1972.

Serial No.: NCI-6295
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Census: A Remote Access Patient Information Storage and Retrieval System

Previous Serial Number: NCI-6039

Principal Investigators: William L. Robinson
Robert J. Esterhay, Jr., M.D.

Other Investigators: Gerald D. Vermeulen, M.D.

Cooperating Units:
Section of Neurological Surgery, NCI, BCRC
Nursing Service, NCI, BCRC
Section of Microbiology, NCI, BCRC
Patient Care Pharmacy Service, NCI, BCRC
Administrative Office, NCI, BCRC

Man Years:

Total:	2
Professional:	2
Other:	0

Project Description:

Objectives:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible, and maintained by a single non-technical individual.

2. To generate a daily report on all inpatients and their locations, along with their vital statistics and current therapy data, to be distributed to all involved in the tasks of clinical research, patient care and administration.

3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

Methods Employed:

1. Information is compiled and recorded daily in a data input log by a medical secretary.

2. A computer program accepts the input datum from the secretary, checks for logical content and accuracy, and records it on the data base.

3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.

4. The report is then distributed to the scientific and professional staff, remote contracting laboratories, and administrative office.

5. The programs and data base are executed and stored on a commercial time-sharing service.

6. Mass memory disk files and remote terminals form the storage and access mediums.

Major Findings:

The Daily Census Report has been utilized by all sections and services of the Cancer Center.

1. Clinical Associates: Used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.

2. Admitting Officer: Used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.

3. Nursing Service: Used to assign patients to vacant beds.

4. Section of Microbiology: Provides the laboratory staff and outside contracting laboratories with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRC. (See Project Report NCI-6039).

5. Patient Care Pharmacy Service: Used to fill the unit drug cart patient trays.

6. The master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings

Major Findings: (Cont'd)

of the patient population which are sorted by status, cancer diagnosis, research protocol, etc.

7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

Significance to Cancer Research:

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Center with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases.

Proposed Course:

Modification of input routines to decrease operator time and provide a fail-safe against hardware failure. Also, development of additional retrieval programs utilizing Census information and information from additional automated data bases at the BCRC.

Honors and Awards: None

Lectures: None

Presentations:

1. William L. Robinson and Robert J. Esterhay, Jr., M.D., Tenth Symposium on Biomathematics and Computer Science in the Life Sciences, Houston, Texas, March 29, 1973, "Census: A Remote-Access Patient Information Storage and Retrieval System".

Publications: None

Serial No.: NCI- 6296
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Problem Oriented List of Charges for Adult Patients with Acute Non-Lymphocytic Leukemia

Previous Serial Number: None

Principal Investigators: Robert J. Esterhay, Jr., M.D.
Victor G. Vogel

Other Investigators: Peter H. Wiernik, M.D.
Clarence L. Fortner, Pharmacist

Cooperating Units: Patient Care Pharmacy Service

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To couple charges in the medical record to the problem under which they are incurred so that for any particular problem the total charge can be assessed.

Methods Employed:

The Problem Oriented Medical Record (POMR) is used to compile a Problem Oriented List of Charges (POLC) for adult patients with acute non-lymphocytic leukemia (ANLL).

Major Findings:

The answers to increase in costs can be supplied by clearly coupling, in the original medical record, the reasons for, the charges for, and the results of medical care.

Significance to Cancer Research:

Current rising costs of medical care and clinical research require that reasons for charges be clearly defined. Such an explanation cannot be provided by the present system of source oriented medical records and source oriented list of charges.

Proposed Course:

Continue present study.

Honors and Awards: None

Publications: None

Serial No.:NCI- 6297
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Streptozotocin Therapy for Malignant Carcinoid and Pancreatic Islet Cell Tumors.

Previous Serial Number: None.

Principal Investigators: Michael J. O'Connell, M.D.
Peter H. Wiernik, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To evaluate the activity of streptozotocin in a Phase II study against malignant carcinoid tumors and pancreatic islet cell tumors.

Methods Employed:

Patients with histologically documented malignant carcinoid tumor or pancreatic islet cell tumor will receive streptozotocin in a dose of 1.5 grams per meter squared on a weekly basis, providing that baseline renal function is adequate.

Major Findings:

This project was activated March 15, 1973 and as of yet there have been no patients entered into the study.

Significance to Cancer Research:

Initial reports appearing in the literature suggested a very high order of activity for streptozotocin against both pancreatic islet cell tumors and malignant carcinoid tumors. Our initial experience suggested a less optimistic

Significance to Cancer Research:(Cont'd)

outlook with regard to metastatic carcinoid tumors. Therefore, this project was undertaken to further define the role of streptozotocin in the therapy of these disorders.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications: None

Serial No.:NCI- 6298
1. BCRC
2. Section of Medical Oncology
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Streptozotocin in the Therapy of Lymphoproliferative Disorders.

Previous Serial Number: None

Principal Investigators: Michael J. O'Connell, M.D.
Peter H. Wiernik, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

1. To evaluate the activity of streptozotocin against various lymphoproliferative malignancies.
2. To compare the relative efficacy of a weekly vs. a cyclical daily dosage schedule.

Methods Employed:

Patients with histologically documented Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphatic leukemia, or chronic lymphatic leukemia are eligible for study regardless of previous therapy provided the pre-treatment renal function is adequate. Patients are randomly assigned to either regimen A or regimen B. Regimen A consists of 5 consecutive daily doses of streptozotocin in a dose of 500 mg/M^2 to be repeated every 2 weeks. Regimen B prescribes weekly doses of streptozotocin at a dosage level of 1.5 grams/M^2.

Major Findings:

This protocol was activated March 1, 1973 and as of yet there have been no entries into the study.

Significance to Cancer Research:

Preliminary clinical trials have suggested that streptozotocin has activity against a variety of lymphoproliferative disorders. Since this agent is not significantly myelosuppressive, it may find a role in the management of these disorders when patients either become refractory to conventional modes of therapy or are unable to tolerate the myelosuppression resulting from such therapy. Therefore, this study was designed to more clearly define the spectrum of activity against patients with a variety of lymphoproliferative disorders.

Proposed Course:

Continue present studies.

Honors and Awards: None.

Publications: None.

Serial No.: NCI-6299
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Therapy of Patients with Metastatic Sarcoma

Previous Serial Number: None

Principal Investigator: William H. Greene, M.D.

Other Investigators: Robert S. Benjamin, M.D., Richard Kaplan, M.D., Peter Wiernik, M.D., Michael Walker, M.D., William Henson

Cooperating Units: None

Man Years

Total:	1/4
Professional:	1/4
Other:	0

Projective Description:

Objectives:

A) Adriamycin vs. Adriamycin + Streptozotocin for Previously Untreated Patients with Metastatic Sarcoma - Metastatic sarcoma forms a considerable portion of death due to malignancy among young people and has largely been more than sporadically responsive to chemotherapy, excluding Ewing's sarcoma and childhood embryonal rhabdomyosarcoma. In recent years adriamycin has proven to be an effective agent in this class of tumors achieving response rates of 25-40% with acceptable mucosal and bone marrow toxicity. In Phase II studies in this Center, streptozotocin, a drug without appreciable myelosuppressive action, has also shown definite anti-sarcoma activity. Therefore, a randomized trial has been adopted comparing adriamycin alone to adriamycin + streptozotocin in previously untreated patients with metastatic sarcoma and without evidence of central nervous system involvement.

Attainment of complete remission or stabilization of partial remission will allow the patient then to be randomly assigned to either weekly maintenance therapy with 5-fluorouracil or no maintenance, until relapse is evident. Patients who fail this trial or who respond and then are not re-inducible with adriamycin + streptozotocin, or who present with or develop evidence of CNS metastases will be treated with part B of this project.

The primary objective of this study is to attain more adequate therapy of metastatic sarcoma without unacceptable bone marrow toxicity. Secondary objec-

Objectives (cont'd):

tives are to continue to demonstrate the efficacy of streptozotocin in sarcoma, and to test the value of 5-fluorouracil as a maintenance phase agent.

B) Intravenous Methyl-CCNU for Advanced Sarcoma - We are in the process of initiating a Phase II protocol for the study of the efficacy of methyl-CCNU administered intravenously in sarcomas. On a Phase I study just completed (see separate project report on IV Methyl-CCNU), an encouraging number of responses occurred in a small group of patients who had failed to respond to, or stopped responding to, adriamycin.

Patients who have failed or progressed on the adriamycin + streptozotocin protocol, and patients who present initially with central nervous system metastases of sarcoma, will receive IV Methyl CCNU in doses from 130-170 mg/M^2 depending on prior therapy. The drug will be repeated each six weeks.

If tolerated, the drug will be continued until relapse occurs.

If the high response rate in sarcomas is confirmed, a randomized clinical trial of Methyl CCNU (IV) vs. other standard therapy, will be undertaken.

C) Assessment of Bone Marrow Reserves and Delayed Hypersensitivity in Patients with Sarcoma - Measurement of serial bone marrow granulocyte mobilization using etiocholanolone in patients being treated with recurrent doses of single agents will perhaps allow assessment of subclinical cumulative bone marrow toxicity.

The serial evaluation of delayed hypersensitivity by skin testing with recall antigens and contact sensitization with dinitrochlorobenzene within a group of sarcoma patients treated in a uniform manner will allow some estimate of the relationship, if any, between intact cell-mediated immunity, extent of disease and prognosis.

Major Findings:

A) Adriamycin vs. Adriamycin + Streptozotocin - The study has only recently (4/1/73) been activated and only two patients have thus far been treated. No evaluation is possible at present.

B) Intravenous Methyl-CCNU for Advanced Sarcoma - Since the study has just been instituted, an evaluation of the findings is not possible.

C) Assessment of Bone Marrow Reserves and Delayed Hypersensitivity in Patients with Sarcoma - As in (A).

Proposed Course: Continue all studies.

Significance to Cancer Research: These studies represent a concerted effort to advance the therapy of metastatic sarcoma by taking advantage of non-overlapping toxicity in clinically active agents to initiate combination chemotherapy. Moreover, it is hoped that more accurate prognosis and assessment of toxicity will be gained via the ancillary studies.

Honors and Awards: None

Presentations:

I Dr. Greene

Proposed Additional Studies in Sarcoma, 4th Conjoint Meeting of the Status of Clinical Studies at the National Cancer Institute, Annapolis,Maryland, January, 1973.

Serial No.: NCI-6900
1. BCRC
2. Medical Oncology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radiation Alone vs. Radiation Therapy Followed by Combination Chemotherapy in the Treatment of Hodgkin's Disease

Principal Investigators: Peter H. Wiernik, M.D., Michael J. O'Connell, M.D.

Other Investigators: Kirkland C. Brace, M.D., Roger Byhardt, M.D.

Cooperating Units: Radiotherapy Service, BCRC

Man Years: (July 1, 1972-June 30, 1973)

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

To determine whether the addition of combination chemotherapy (MOPP) administered following radiation therapy to all involved lymph node regions as determined by staging laparotomy is superior to radiation therapy alone of involved plus adjacent lymph node regions in patients with Hodgkin's disease. This study was designed to compare in a randomized fashion the two best arms of our previous project (NCI-6230).

Methods Employed:

Previously untreated patients with less than Stage IIIB Hodgkin's disease on the basis of exploratory laparotomy were randomized to receive either radiation therapy alone to involved plus adjacent lymph node regions, or radiation therapy to involved lymph node regions only followed by combination chemotherapy (MOPP) by card selection. Patients with Stage I or II disease were randomized separately from patients with Stage III disease to insure roughly equal distribution of therapeutic modality employed for patients with apparently localized disease and generalized nodal disease respectively.

Major Findings:

To date, 26 patients with Stage I or II disease have been randomized to receive radiation alone, and of this group 5 have relapsed. On the other hand, of the 19 patients with Stage I or II disease randomized to receive radiation therapy followed by chemotherapy, there has been only one relapse. Fourteen patients with Stage IIIA disease were randomized to receive radiation alone

Major Findings (cont'd):

and one has relapsed. Seventeen patients with Stage IIIA disease received radiation therapy followed by chemotherapy with one relapse observed. There have been 4 deaths, one in the radiation therapy/chemotherapy group and 3 in the radiation alone group.

Significance to Cancer Research:

The preliminary data presented suggest a lower recurrence rate in the group of patients receiving radiation therapy followed by chemotherapy. However, there is no significant difference in survival rates at this point and it is not yet certain that the potential risk from drug toxicity when combination chemotherapy is administered following radiation is accompanied by an increased survival rate.

Proposed Course:

Additional patients are required for each arm of the study and longer follow-up periods are necessary before firm conclusions as to the relative efficacy of combined radiation therapy and chemotherapy as compared to radiation therapy alone in the management of Hodgkin's disease can be made.

Honors and Awards: None

Publications: None

Presentations:

Dr. Wiernik

1. Combined Modality Therapy for Hodgkin's Disease. Case-Western Reserve University, School of Medicine, Cleveland, Ohio, February 18, 1973.

2. What is the Proper Therapy for Localized Hodgkin's Disease? University of Maryland School of Medicine, Baltimore, Maryland, January 21, 1973.

Serial No.: NCI-6024
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of a Neurosurgical Service with Primary Interest in the Treatment of Brain Tumors at the BCRC, NCI

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Richard Kaplan, M.D.
Donald Sklansky, M.D.
Howard Weiss, M.D.
Marshall Levine, M.D.

Cooperating Units: Medical Service, BCRC, NCI
Department of Surgery, USPHS Hospital
Department of Neurology, USPHS Hospital
Baltimore, Maryland

Man Years:

Total:	1.5
Professional:	1.5
Other:	

Project Description:

Objectives:

This ongoing project is designed to develop an effective research neurosurgical service to evaluate various modalities of treatment of intracranial neoplasm. Pilot studies are performed to evaluate the efficacy of drug in relation to Phase I - II and neuropharmacologic studies. Neurosurgical consultation and expertise are provided the USPHS Hospital, Baltimore, and diagnostic and therapeutic services are provided. Consultation and supervision of NCI projects related to the treatment of malignancy within the intracranial space are made available. Interdisciplinary studies of blood brain barrier, pharmacology and cellular membrane permeabilities are undertaken.

Methods Employed:

Patients with diagnosed intracranial neoplasm are referred to the BCRC, Section of Neurological Surgery, for evaluation and

chemotherapy both from within the private community, as well as the PHS system. Primary unoperated upon CNS tumor is preferred, so that pharmacologic evaluation of chemotherapeutic agents, intracranial markers, and specific and non-specific humoral indicators of malignancy may be carried out as part of the operative procedure. Complete care on an out-and-inpatient basis, as well as utilization of the intensive care unit and blood component therapy services are available. Due to the potential toxicity of drugs employed, close monitoring of patients is a necessity. Studies utilizing oral and a newly developed intravenous preparation of Methyl CCNU and Epipodophyllotoxin (PTG, VM-26) have been undertaken. This service has also participated in the Brain Tumor Study Group.

Frequent consultations and neurologic evaluation of BCRC patients are provided the medical staff to assist in the general medical oncology program. A particular interest in CNS leukemia, as well as metastasis of other tumors, is pursued. CSF clearance of inert substances has been carried out.

In consultation with the Departments of Medicine, Surgery and Neurology of USPHS Hospital, Baltimore, neurosurgical evaluation, neurodiagnostic procedures and surgical intervention for general neurosurgical disease are provided. Consultations on difficult diagnostic problems are undertaken. Thru the Director, surgical and parasurgical communications are maintained. Formal and informal consultations are provided to the greater Baltimore medical community in relation to brain tumor therapy. Informal discussions, telephone consultations and formal lectures are given.

Major Findings:

During the fiscal year, there were 110 neurosurgical admissions and 424 out-patient visits. A Phase II study of oral Methyl CCNU has been completed and demonstrated 8 satisfactory responses out of 19 evaluable patients with primary malignant brain tumor. A similar Phase II study of PTG has provided a remission rate and duration similar to that obtained with the nitrosoureas but with less marrow suppressant toxicity. A Phase I toxicology and pharmacologic study of a newly developed intravenous preparation of Methyl CCNU has been completed and established the dosage level of 170 mg/m^2 in previously untreated patients (approximately 1/2 the tolerated oral dose).

The lack of systematized criteria and truly measurable disease in patients with primary brain neoplasm has long thwarted therapeutic evaluation. A CNS grading system based upon symptoms and function as well as neurologic examination has been developed

to provide a numerical grade for the patient during the course of his disease. Factors other than therapeutic response which may affect the staging system have been consistently dealt with. The CNS disease staging system is continuing to be evaluated.

Consultation services within the hospital, the city of Baltimore, nationally and internationally have been provided. Lectures, papers and discussions have been undertaken within the neuroscience community in order to effectively bring out the Division of Cancer Treatment's programs.

Significance to Biomedical Research and Program of the Institute:

The incident of central nervous system neoplasm exceeds that of Hodgkin's Disease. With increasingly successful therapy of cancer elsewhere in the body, cerebral metastasis is becoming a more important obstruction to conquest of these diseases. CNS leukemia illustrates this problem well as it may be the sole sanctuary of the disease which prevents its cure. Development of a study unit to evaluate the efficacy, toxicity and neuropharmacology of drugs used to treat these disease processes is essential to future control of cancer. Development of therapeutic and toxicologic data related to the treatment of primary brain tumor from the utilization of the CNS staging system provides an objective scale for the more reliable analysis of data.

Proposed Course:

This service will continue to admit, diagnose and treat operatively and chemotherapeutically and radiotherapeutically patients with intracranial malignancy. Phase I studies of new chemotherapeutic agents with appropriate pharmacokinetic characteristics for successful brain tumor chemotherapy will be undertaken. Phase II studies of intravenous Methyl CCNU alone and in combination for brain tumors and for sarcomas will proceed, as well as investigation of intravenous Methyl CCNU in addition to PTG. Continued participation in the Brain Tumor Study Group will be undertaken and consultation services, as described above, will also continue.

Honors and Awards: None

Publications:

Walker, M.D.: BCNU and X-ray Extend Survival in Brain Tumors. Infectious Dis. 2, 13: 20, Sept. 15, 1972.

Walker, M.D.: Brain Tumors, in Cancer Medicine, edited by J. F. Holland & E. Frei, III, Philadelphia, Lea & Febiger (in press)

Rosenblum, M.L., Bowie, D.L., and Walker, M.D.: Diffusion of 1-(2-Chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (Methyl CCNU) from Silicone Rubber Capsules, In Vitro and In Vivo: A Potentially New Mode of Chemotherapy Administration (in press) Cancer Research

Walker, M.D.: Physiological Barriers to Pharmacologic Efficacy. Proceedings of 5th International Congress on Pharmacology. (in press)

Walker, M.D.: Chemotherapy of Malignant Glioma. Proceedings of the Seventh National Cancer Conference. (in press)

Walker, M.D.: Nitrosourea in Central Nervous System Tumors Cancer Chemother. Rep. (in press)

Shepard, R.F., Walker, M.D. and Rogers, W.I.: Cerebrospinal Fluid (CSF) Perfusions with Selected Anticancer Agents in the Rhesus Monkey. Proceedings 13: 46, May 1972.

Walker, M.D. and Gehan, E.A.: An Evaluation of 1-3-Bis(2-Chloroethyl)-1-Nitrosourea (BCNU) and Irradiation Alone and in Combination for the Treatment of Malignant Glioma. Proceedings 13: 67, May 1972.

Young, R., Canellos, G., Schein, P., Chabner, B., Walker, M. and DeVita V.: Initial Clinical Trials with Methyl-CCNU 1-(2-Chloroethyl)-3-(4-Methylcyclohexyl)-1-Nitrosourea (MeCCNU). Proceedings 13: 101, May 1972.

Walker, M.D., Mann-Kaplan, R.S., Sklansky, D.B., Levine, M.H., Weiss, H.D., and Wiernik, P.H.: Toxicity and Pharmacology of Intravenous Methyl CCNU (IV MeCCNU) in Man. Proc. Am. Assoc. Cancer Res. 14: 39, March 1973.

Sklansky, B.D., Mann-Kaplan, R.S., Reynolds, Rosenblum, M.L., and Walker, M.D.: 4'Demethyl-Epipodophyllotoxin -β-D Thenylidene Glucoside (PTG) in the treatment of Intracranial Neoplasms. Proceedings Ninth Annual Scientific Meeting of the American Society of Clinical Oncology, April 1973.

Merker, P.C. and Walker, M.D.: Behavioral and Neurotoxicological Effects of Anti-Cancer Drugs Perfused into the Brain of Monkeys. European Society for the Study of Drug Toxicity Meeting, June 1973, Zurich, Switzerland.

Presentations: Serial No.: NCI-6024

Seventh Joint Meeting of the Clinical Society and Officers Association of the USPHS, New York, and Chairman of Session on "Advances in Cancer Therapy." May 31, 1972.

National Conference on Cancer Chemotherapy, New York. Talk on "Central Nervous System Cancer." June 1-3, 1972.

Radiation Therapy Oncology Group Meeting, Philadelphia, Pa. Talk on "Brain Tumors (with Brain Tumor Study Group)." June 16, 1972.

Fifth International Congress on Pharmacology Meeting, San Francisco, Calif. Talk on "Physiologic Barrier to Pharmacologic Efficacy." July 25, 1972.

Talk to the MacQueen Gibbs Willis School of Nursing. "Function and Research Program of the Baltimore Cancer Research Center." August 1, 1972.

Seventh National Cancer Conference, Los Angeles, Calif. Talk on "Chemotherapy of Malignant Gliomas." September 29, 1972.

Congress of Neurological Surgeons, Denver, Colo. Moderator of Special Interest Workshop on "Biology and Chemotherapy of Brain Tumors." October 18, 1972.

Late Effects Workshop, Boston, Mass. Talk on "Effects of Chemotherapy on the Central Nervous System." October 20, 1972.

Talk to the St. Joseph's Hospital School of Nursing on "Malignant Brain Tumors - Diagnosis and Treatment." October 31, 1972.

Appearance on the Allan Christian Show, WCBM Radio 3 hour radio show with call in questions from listeners, plus information to public on cancer. December 8, 1972.

Medical Record Librarian School lecture on "Surgery of Central Nervous System." December 19, 1972.

Baltimore Neurological Society, Johns Hopkins Hospital, Baltimore, Maryland: "Recent Study in the Treatment of Malignant Glioma." January 17, 1973.

University of Maryland School of Medicine: "Recent Advances in Treatment of Malignant Gliomas," at the Radiation Research Seminar. January 23, 1973.

Research Society of Neurological Surgeons, San Diego, California: Chemotherapy and Brain Tumor. February 16, 1973.

Serial No.: NCI-6024

American Society of Clinical Oncology, Atlantic City, New Jersey: 4'Demethyl-Epipodophyllotoxin -β-D-Thenylidene Glucoside (PTG) in the Treatment of Intracranial Neoplasms. April 10, 1973.

American Association of Cancer Research, Atlantic City, New Jersey: "Toxicity and Pharmacology of Intravenous Methyl CCNU (IV MeCCNU) in Man." April 12, 1973.

Serial No.: NCI-6202
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Murine Intracerebral Melanoma Study

Previous Serial Number: Same

Principal Investigator: Donald S. Bowie, B.S.

Other Investigators: Michael D. Walker, M.D.
John Hilton, D. Phil.

Cooperating Units: None

Man Years:

Total: .3
Professional:
Other: .3

Project Description:

Objectives:

The major objectives of this project are to continue the study of the efficacy of MeCCNU against the murine intracerebral melanoma, and the interaction of various drugs with this chemotherapeutic agent.

Methods Employed:

B-16 melanoma of the Jackson Laboratory, Bar Harbor, Maine, has been employed for serial transplant. Varying doses of IV-MeCCNU have been used. Before MeCCNU was given animals were pretreated with saline, phenobarbitol, SKF-525 A, decadron and dilantin.

Major Findings:

MeCCNU at 30 mg/kg (LD_{10}) increased the life span of intracranial bearing B-16 mice 70% compared to untreated controls. Animals pretreated with phenobarbitol, dilantin and decadron had an increase in life span of 30-43%. SKF-525 A pretreated animals had an increase in life span of 114%.

Proposed Course:

Further studies will be pursued using MeCCNU against other drugs in this mouse system. Studies on the effect of MeCCNU upon DNA synthesis will be undertaken.

Honors and Awards: None

Publications: None

Serial No.: NCI-6203
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Drug Distribution in Patients Receiving Therapeutic Doses of CCNU. (Phase II)

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: V.T. Oliverio, Ph.D., V.T. DeVita, M.D.
M.L. Rosenblum, M.D. and A.F. Reynolds,M.D.
Donald M. Bowie, B.S.

Project Descripton: Project Terminated.

Serial No.: NCI-6204
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Toxicity of Intracerebral Chemotherapy in Monkeys

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Dan Dalgard, D.V.M.

Cooperating Units: Hazelton Laboratories, Falls Church, Va.

Man Years:

Total:	1.0
Professional:	.5
Other:	.5

Project Description:

Objectives:

With the increase in meningeal leukemia and the interest in neoplasms within the central nervous system, the use of intrathecal chemotherapy is expected to increase. In this ongoing project, a relatively standardized technique for evaluating intrathecal chemotherapy in monkeys is being employed.

Methods Employed:

Intracisternal installation of carefully chosen drugs are first performed to determine acute immediate toxicity. From this information dose levels for chronic studies simulating the dose schedules used in man are developed. Evaluation of activity in indices, hematologic parameters and cerebral spinal fluid are made. Histopathologic review is performed on the upper cervical cord, brain stem and brain.

Major Findings:

Evaluation of Thiotepa has defined 6mg/m^2 intrathecally twice weekly for 4 weeks as the toxic dose low and 12mg/m^2 intrathecally twice weekly for 4 weeks as the toxic dose high. The lethal dose

was felt to be 24mg/m^2 or greater. On the basis of this data, a protocol has been designed to evaluate the use of Thiotepa in the treatment of resistant meningeal disease. Due to the concern related to the preservatives Methyl, parabenes and proparabenes in methotrexate, a prolonged study utilizing 10 times the amount normally found in man has been conducted. Evaluation of these animals indicates no significant finding from these preservatives. Specially prepared hydroxyurea (neutral and without preservatives) has been evaluated at 25, 50 and 100mg/kg twice weekly for 4 weeks without evidance of toxicity.

Significance to Biomedical Research and Program of the Institute:

Intrathecal chemotherapy for human neoplasms has been limited to methotrexate, cytosine arabinoside and the current study to evaluate intrathecal Thiotepa. As the control of cancer in the periphery becomes greater, so do the chances of developing CNS metastasis. Therefore, developing appropriate toxicologic information concerning drugs placed in the CSF pathways will provide necessary information for expanding intrathecal drug usage.

Proposed Course:

Additional drugs will be added to the study this year as well as a more careful evaluation of previously investigated drugs. In conjunction with the Laboratory of Pharmacology, pharmacologic distribution studies will be undertaken in the monkey so as to more clearly understand the effects seen in this animal.

Honors and Awards: None

Publications: None

Serial No.: NCI-6268
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radio-labeled Distribution of Methyl CCNU in Dogs

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Donald M. Bowie, B.S.
John Hilton, D. Phil.

Cooperating Units: Laboratory of Toxicology, DCT, NCI

Man Years:

Total:	.2
Professional:	.1
Other:	.1

Project Description:

Objectives:

This project is designed to determine the pharmacologic characteristics of Methyl CCNU in the dog as preparation for similar determinations in humans. Since Methyl CCNU is aqueous and insoluble, a parental formulation technique is being developed and evaluated in the dog.

Methods Employed:

Following intravenous or intragastric administration of C^{14} MeCCNU labeled in the chloroethyl and methylcyclohexyl moieties, serial blood, CSF, urine, bile, muscle and brain samples have been obtained from 4 minutes up to 24 hours. Direct sample counting, as well as extraction and chromatography, have been carried out. Utilizing different substances pharmacologic acceptability of various vehicles is determined. Fat emulsion appears satisfactory for deliverence of this drug. Comparable pharmacologic evaluation of the emulsifiers is also being undertaken.

Major Findings:

Besides pharmacologic data generated during the previous year, toxicologic evaluation of intravenous Methyl CCNU has been undertaken indicating the lethal dose, toxic dose high, toxic dose low and highest non-toxic dose as being at 280, 185, 125, and 82mg/m^2 respectively. On the basis of this a human Phase I study has been undertaken. Further comparative pharmacology in the dog awaits the preparation of the methyl-labelled material.

Significance to Biomedical Research and Program of the Institute:

Methyl CCNU is one the newest of the nitrosourea compounds and heretofore could only be delivered orally due to its lipid insolubility. Preparation of an intravenous dosage system, development of pharmacodynamics and toxicity within the dog have permitted the rapid Phase I evaluation of this material in man.

Proposed Course:

This project awaits the completion of the methyl-labelled MeCCNU. During the interim, the active metabolite and the effects on polymerase will be investigated.

Honors and Awards: None

Publications: None

Serial No.: NCI-6282
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Brain Tumor Study Group

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Mathilde Solowey, Ph.D., Linda Stinson, and Members of the BTSG

Cooperating Units: Members of the BTSG

Man Years:

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description:

Objectives:

The Brain Tumor Study Group was established in order to provide a rapid, scientific and controlled method of evaluating chemotherapy in the treatment of brain tumors utilizing Phase III type studies. The program has been broadened to include evaluation of radiation therapy as a modality alone and in combination with chemotherapy. Additionally, the group is also undertaking Phase II studies in order to make the selection of chemotherapeutic agents more meaningful.

Methods Employed:

Members of the Brain Tumor Study Group have established a cooperative ongoing continuous effort providing newly-operated upon patients for randomization to protocol studies. One Phase III protocol study terminated for accrual of patients, evaluated BCNU and radiation alone and in combination in a prospective, controlled and randomized study. Another Phase III protocol study is evaluating Methyl CCNU alone and in combination with radiation, radiation alone, and BCNU in combination with radiation.

This year, the program has been broadened to include the investigation of four new drugs in Phase II protocol studies. Adriamycin, Dibromodulcitol, Procarbazine, and Streptozotocin will be evaluated in a paired, randomized study. Patients deemed failures to therapy with one of the aforenamed drugs are to be rerandomized to either a high tapered dose of corticosteroid, or a moderate prolonged course of corticosteroid therapy. Administrative support is provided by the Central Office, and institutional support by the contract mechanism. Meetings of the Brain Tumor Study Group, at quarterly intervals, staff visits from the Central Office provide appropriate communication and exchange of information.

Major Findings:

The BCNU protocol (#69-01) has had approximately 302 patients randomized to four arms. Current evaluation tends to confirm the preliminary finding that BCNU and radiation are superior modes of therapy compared to untreated patients, and that combined BCNU and radiation therapy is superior to either therapy used alone. Detailed analysis of the studies is under way.

The BTSG held its first multidisciplinary meeting to bring together neurosurgeons, radiotherapists and neuropathologists of the participating institutions, so as to provide a focus for inter and intrainstitutional communication. This meeting was consistent with the broad mission of the DCT in developing multidisciplinary cooperative programs. During the past year, VA participation in the BTSG program has been activated and it is anticipated that case accrual will increase the scope of Phase II studies, particularly, but also Phase III studies.

Significance to Bio-medical Research and Program of the Institute:

These studies are the only prospective, controlled, randomized studies performed in the United States in the treatment of brain tumor. They provide specific detailed and timely information concerning the efficacy of various therapeutic modalities for malignancy of the central nervous system.

Proposed Course:

The BCNU-radiotherapy study has been completed with respect to acquisition of new cases for study. Evaluation of results is in progress. A new Phase III protocol evaluating Methyl CCNU, BCNU and irradiation in various combinations has been activated and case acquisition is progressing satisfactorily. Phase II studies evaluating Adriamycin, Dibromodulcitol, Procarbazine and Streptozotocin are activated and accruing patients.

The BTSG will continue its studies over this year, seek new members with appropriate qualifications for participation, and attempt to define applied research projects of significance in the treatment of malignant central nervous system tumor.

Honors and Awards: None

Publications:

Walker, M.D. and Gehan, E. A.: An Evaluation of 1-3-Bis(2-Chloroethyl)-1-Nitrosourea (BCNU) and Irradiation Alone and in Combination for the Treatment of Malignant Glioma. Proceedings 13: 67, May 1972.

Serial No.: NCI-6283
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: An Evaluation of 4'Demethyl-Epipodophyllotoxin -β-D Thenylidene Glucoside (PTG) in a Phase II Study Treating Primary Intracranial Neoplasms

Previous Serial Number: NCI-6283

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: B. Donald Sklansky, M.D., Richard Mann-Kaplan, M.D., Howard Weiss, M.D. and Marshall Levine, M.D.

Cooperating Units: Medical Service, BCRC

Man Years:

Total:	.9
Professional:	.9
Other:	0.0

Project Description:

Objectives:

This Phase II study is designed to evaluate the efficacy of PTG in the treatment of primary intracranial neoplasms and provide further toxicologic information concerning multiple doses of this lipid soluble drug.

Methods Employed:

Patients harboring primary intracranial neoplasms were treated with PTG giving multiple courses each consisting of 100-130mg/m^2 administered intravenously weekly for 6-8 weeks. Drug responses were measured by using a standardized central nervous system disease staging system as well as a lumbar puncture brain scan etc. Frequent hematologic and bio-chemical parameters are obtained

Major Findings:

Twenty-three patients with primary intracranial neoplasms were treated with PTG, three were considered unevaluable because they died after receiving considerably less than 1 full course of drug.

Major Findings (con't):

The twenty evaluable patients have received between 1 and 11 courses of drug. Objective neurologic improvement was noted in 4 of 12 patients who had measurable disease at the outset of therapy. The progression-free interval among these 4 remission patients was 7 + months as of January 1, 1973. Among the 8 patients who are without measurable disease at the outset of therapy, the progression-free interval was 15 + months to January 1, 1973. Two of these latter patients progressed prior to January 1, 1973 and 2 have subsequently progressed after that date. Toxicity was mild although consistent anemia and mild leukopenia were observed. Clinically significant marrow toxicity was seen only in 3 patients all of whom had prior extensive chemotherapy.

Significance to Bio-medical Research and Program of the Institute:

Analysis of results indicates PTG to have some efficacy in the treatment of primary intracranial neoplasms with minimal toxicity.

Proposed Course:

A new protocol will be devised which uses PTG in combination with Methyl CCNU.

Honors and Awards: NONE

Publications:

Abstracts of the American Society of Clinical Oncology with formal manuscript submitted to Cancer.

Presentation:

American Society of Clinical Oncology, Atlantic City, April 10, 1973

Serial No.: NCI-6284
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Methyl CCNU Diffusion from Silicone Capsules: In Vitro and In Vivo

Previous Serial Number: SAME

Principal Investigator: Mark L. Rosenblum, M.D.

Other Investigators: Donald M. Bowie, B.S.
Michael D. Walker, M.D.

Cooperating Units: NONE

Man Years:

Total: .1
Professional
Other: .1

Project Description:

Objectives:

This project is designed to determine the diffusion of 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (NSC 95441) Methyl CCNU from silicone capsules in vitro and in vivo, and explore the influence of factors affecting its dynamics.

Methods Employed:

Silicone (dimethylpolysiloxane) capsules are filled with dry crystalline Methyl CCNU to which has been added ^{14}C-chloroethyl label. In vitro studies were performed by suspending the capsules into various fluids, shaking at 37° and sampling at appropriate intervals. The amount of drug diffused is measured by standard isotopic techniques. Capsules were implanted subcutaneously into rats for the in vitro study and the amount remaining after various times analyzed.

Major Findings:

A constant release of Methyl CCNU into the surrounding bath was observed. The diffusion rate decrease over time both in vitro

Major Findings (cont'd)

and in vivo and is proportional to surface area.

Significance to Biomedical Research:

Constant administration of certain chemotherapeutic agents may be advantageous, but at present requires constant IV infusion for constant blood levels. The hazards of long term treatment by that mode is well-known. In addition, very hydrophobic agents may not be given IV at all. Subcutaneous insertion of drug filled silicone capsules may provide a constant diffusion of many agents with minimal hazard.

Proposed Course:

This project is completed and has been prepared for publication.

Honors and Awards: NONE

Publications: Rosenblum, M.L., Bowie, D.L., and Walker, M.D.: Diffusion of 1-(2-Chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (Methyl CCNU) from Silicone Rubber Capsules, In Vitro and In Vivo: A Potentially New Mode of Chemotherapy Administration. Cancer Research , April 1973. (in press)

Serial No.: NCI-6285
1. Baltimore Cancer Research Center
2. Section of Neurological Surgerh
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effect of Polyamines on Rat Brain Nucleic Acid Metabolism

Previous Serial Number: SAME

Principal Investigator: Arden F. Reynolds, Jr., M.D.

Other Investigators: Diane H. Russell, Ph.D.

Cooperating Units: Section of Enzymology, BCRC, NCI

Man Years:

Total .1
Professional: .1
Other:

Project Description:

Objectives:

To study the effects of putrescine, spermidine and spermine on rat nucleic acid metabolism.

Methods Employed:

Rats are injected intracisternally with a polyamine, then re-injected at a later time with tritiated thymidine or uridine. The method of Fujiolea et al, JBC 238, 3401 (1963) is used for measuring uridine or thymidine uptake in rat brain.

Major Findings:

Results indicate a possible stimulatory effect upon brain polyamine synthesis 4 hours after injection intracisternally.

Proposed Course:

This project is terminated and has been prepared for presentation and publication.

Honors and Awards: NONE

Serial No.: NCI-6285

Publications: Reynolds, Arden F. and Russell, Diane, H.: Stimulation of ^{14}C-Uridine Incorporation into RNA by intracisternal Injection of Putrescine. Fed. Proceedings 32, 429, 1973.

Presentation: To be presented to the American Physiological Society, April 18, 1973.

Serial No.: NCI-6286
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Methyl CCNU: Phase I Toxicologic and Neuropharmacologic Evaluation and Phase II Therapeutic Study

Previous Serial Number: Same

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Richard Kaplan, M.D.
B. Donald Sklansky, M.D.
Peter H. Wiernik, M.D.
Howard Weiss, M.D.
Marshall Levine, M.D.
Donald L. Bowie, B.S.
John Hilton, D. Phil.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	1.1
Professional:	.9
Others:	.2

Project Description:

Objectives:

Pharmacologic investigation of this latest nitrosourea is continued, while a Phase II study of therapeutic efficacy in malignant glioma and tumor metastatic to the central nervous system is initiated.

Methods Employed:

Patients with progressive central nervous system symptoms, after appropriate surgery, radiotherapy or chemotherapy, are referred to the Neurosurgical Section, Baltimore Cancer Research Center, NCI, for treatment with newer chemotherapeutic agents with the potential for crossing the blood-brain barrier. A number of patients of the Medical Service, BCRC, NCI with metastatic tumor involvement of the central nervous system are also referred for study.

Methyl CCNU is administered by the oral route in optimal doses as previously established in the Phase I of this study. These are 290 mg/m^2 in previously untreated patients, and 220 mg/m^2 or lower in those patients with bone marrow compromise from previous therapy, tumor infiltration, etc. Careful monitoring of hematologic and toxicologic parameters is obtained and the patients are supported with blood products, platelets and white cells required. Select patients receive, along with their therapeutic dose of Methyl CCNU, radiolabelled tracer doses for pharmacologic study. Blood, urine, cerebral spinal fluid and normal and abnormal tissues are analyzed for radioactivity, and drug and metabolite content.

Major Findings:

The dose of 290 mg/m^2 in previously untreated patients is confirmed and further pharmacologic studies have revealed no important exceptions to the pharmacology as previously described.

Thirty-two patients were therapeutically evaluable. Twenty-seven of these patients had intracranial tumors, of which 23 were primary and 4 were metastatic. Of the 23 patients with primary brain tumors, 19 of them had adequate trials with the drug, that is at least one full course at what has been established as adequate dosage. Sixteen of these patients had malignant gliomas, 3 of them were without measurable disease at the initiation of chemotherapy, and all 3 have remained well from 18 weeks to more than 1 year after beginning MeCCNU. Thirteen patients had measurable neurologic dysfunction, and 3 of these achieved a partial remission lasting from 16 to more than 60 weeks. Of the 19 patients with primary brain tumors, 8 have had satisfactory responses to MeCCNU lasting 16 to 60+ weeks. Of the 5 patients who did not have intracranial tumors, there was only 1 short-lived response in a patient with a reticulum cell sarcoma.

Significance to Biomedical Research and Program of the Institute:

The nitrosoureas are among the most active of the antineoplastic compounds available. Preliminary studies would indicate that the level of therapeutic response to orally MeCCNU is equivalent to that seen with BCNU. Determination of tolerable dose, toxicity, pharmacologic characteristics, and predictive parameters will allow more intelligent and objectively founded choice of therapeutic regimen and delivery of that regimen in the future.

Proposed Course:

The Phase II study of all MeCCNU will be promptly completed this year and the Phase III BTSG study employing this drug will be followed.

Honors and Awards: None

Publications: None

Presentations: National Cancer Institute, Division of Cancer Treatment, New Drug Liaison Committee Meeting, January 22, 1973.

Serial No.: NCI-6901
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Intravenous Methyl CCNU Phase I Study

Previous Serial Number: None

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Richard Kaplan, M.D.
B. Donald Sklansky, M.D.
Howard D. Weiss, M.D.
Marshall A. Levine, M.D.
Peter H. Wiernik, M.D.

Cooperating Units: Medical Oncology Service, BCRC, NCI

Man Years:

Total:	.7
Professional:	.7
Other:	

Project Description:

Objectives:

Methyl CCNU is the newest of the nitrosourea compounds shown to have marked activity in a variety of tumor systems, notably the Lewis lung. Phase I and II studies with MeCCNU have been completed utilizing the oral preparation. The development of a parenteral delivery system has allowed a Phase I study of IV Methyl CCNU in man.

Methods Employed:

Using 5% ethanolic solution in 10% fat emulsion, patients were treated at increasing doses of IV MeCCNU (45,90,130,150 and 170 mg/m2) until the maximally tolerated dose was determined. Patients were entered into the Phase I study in coordination with the Medical Oncology Service, BCRC, and carefully monitored for hematologic and toxicologic parameters. Selected patients received, along with their therapeutic dose of MeCCNU, radiolabeled tracer doses for pharmacologic distribution stiduies. Blood, urine, cerebral spinal fluid and normal and malignant tissues, when accessible, were analyzed for radioactivity, drug and metabolic content.

Major Findings:

IV Methyl CCNU was tolerated in doses up to 170 mg/m2 in previously untreated patients. Patients receiving extensive prior therapy (irradiation or chemotherapy) should increased sensitivity, and doses of 130 mg/m2 were considered the maximally tolerated dose. In previously untreated patients, the hematologic toxicity is moderate reversible thrombocytopenia occurring in all patients receiving 150 mg/m2 or more. Leukopenia was less consistent and rarely was the nadir below 2,000. No renal or hepatic dysfunctions were noted.

Pharmacologic distribution studies indicated peak plasma radioactivity at the end of the 1 hour infusion with an initial half-life of 1 hour, a second component of approximately 12 hours, and a prolonged half-life of 3 1/2 days. Approximately 40% was bound to plasma proteins consistently throughout the study. Between 50 and 70% of administered radioactivity is excreted in the urine over 5 days. Only 14 and 18% of plasma concentrations at 1 hour and 4 hours respectively appeared in the spinal fluid.

Preliminary responses have been noted in patients with malignant gliomas and in patients with sarcoma. A more detailed study is to follow.

Significance to Biomedical Research:

BCNU is the only member of the nitrosourea family with sufficient solubility characteristics to be administered intraven ously. The development of a method of administration of Methyl CCNU has permitted controlled delivery of the drug, development of pharmacologic characteristics and completion of a Phase I study indicating a maximally tolerated dose of 170 mg/m2.

Proposed Course:

The Phase I study of IV Methyl CCNU has been completed. Phase II studies employing this drug and mode of delivery have been developed for the treatment of both malignant gliomas and sarcomas. Further definition of the toxicity parameters will be undertaken and pharmacologic characteristics more clearly defined.

Honors and Awards: NONE

Publications:

Publications (cont'd)

Walker, M.D., Mann-Kaplan, R.S., Sklansky, D.B., Levine, M.H., Weiss, H.D., and Wiernik, P.H.: Toxicity and Pharmacology of Intravenous MeCCNU (IV MeCCNU) in Man. Phase I. Proceedings 14: 39, 1973.

Presentations:

American Association for Cancer Research, Atlantic City, N. J., April 12, 1973. Toxicity and Pharmacology of Intravenous MeCCNU (IV MeCCNU) in Man. Phase I.

Serial No.: NCI-6904
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the Anti-Tumor Activity of Methyl CCNU and Related Compounds at the Subcellular Level

Previous Serial Number: None

Principal Investigator: John Hilton, D. Phil.

Other Investigators: Michael D. Walker, M.D.
Donald L. Bowie, B.S.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	.8
Others:	.3

Project Description:

Objectives:

To determine the site of action of Methyl CCNU and related compounds as tumor growth inhibitors. This will allow a judicious choice of co-inhibitors used in combination chemotherapy.

Methods Employed:

The nitrosourea induced rat glioma used in these studies is maintained in culture. Cell suspensions are introduced stereotactically into the parietal white matter or subcutaneously in the flank of male rats. Methyl CCNU and related compounds, suspended in a lipid emulsion, are administered via the tail vein.

Major Findings:

Studies on the antineoplastic activity of Methyl CCNU are in progress using a nitrosourea induced, transplantable rat glioma as the target tissue. The tumor, implanted either intracerebrally or subcutaneously, is susceptible to growth inhibition by MeCCNU. DNA synthesis, measured by radioactive thymidine incorporation,

is the most sensitive macromolecular synthetic process affected by Methyl CCNU. The onset of inhibition of DNA synthesis is rapid (3 hours after Methyl CCNU) and inhibition lasts for about 12 days after treatment. Similar inhibition of thymidine incorporation into DNA by Methyl CCNU is observed in the intracerebrally implanted or flank implanted glioma.

The immediate precursor of DNA thymidine is thymidine triphosphate (TTP). A decrease in the specific radioactivity of the pool of TTP following the administration of labelled thymidine could account for the observed effect of Methyl CCNU on thymidine incorporation into DNA. No abnormality in the pool size or specific activity of TTP commensurate with the inhibition of thymidine incorporation into DNA has been observed in tumor tissue from Methyl CCNU treated animals.

Proposed Course:

To attempt an identification of the step(s) in DNA replication sensitive to Methyl CCNU and related compounds.

Honors and Awards: None

Publications: None

Serial No: NCI-6043
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Automated Monitoring of Drug Therapy

Previous Serial Number: Same

Principal Investigators: Clarence L. Fortner, James K. Hooper

Other Investigators: Michael D. Walker, Richard Hsieh, William R. Grove, Peter H. Wiernik

Cooperating Units: Division of Health Services Research, Baltimore USPHS Hospital; Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 2-1/2
Professional: 1/2
Other: 2

Project Description:

Objectives:

To provide inexpensive, efficient and rapid methods of collecting data concerning the clinical trials of experimental drugs.

Methods Employed:

An automated method of ordering and recording drug therapy was designed and implemented.

An inexpensive pen and paper system of processing drug information provided accurate, periodic summaries of patient's drug therapy. A medicaiton for each of a patient's drugs is marked at the time of drug administration. Drug and patient identifiers are typed on the card and this data is read weekly by optical character recognition. The data is collated and weekly drug summaries are printed listing total dose, frequency of administration or omission of each drug. A drug utilization report is also obtained as a by product.

The physician writes his order directly on the medication card that produces four copies: the medicine card copy, the pharmacy copy, and copies for medical records and automated analysis. A clerk types the drug name, dosage information, patient name and record number and date drug was initiated.

Methods Employed (cont'd)

The drug form has been revised recently to include additional information. The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patients height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered.

Major Findings:

The medication card has proven to fulfill all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by physicians and pharmacists and become a permanent record of drug therapy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart.

Significance to Biomedical Research:

This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

Honors and Awards: None

Publications: None

Serial No: NCI-6236
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove, William A. Cornelis, and Robert De Christoforo

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1/8
Professional: 1/8
Other: 0

Project Description:

Objectives:

To evaluate the feasibility of having a pharmacist interview all patients admitted to the Baltimore Cancer Research Center for the purpose of obtaining a drug and allergy history.

Methods Employed:

The pharmacist questions each patient admitted to the Medicine and Neurosurgical Services, BCRC, concerning current and past medication usage and history of allergy.

A written record of this interview is placed in the history section of the patient's chart.

Major Findings:

Pharmacists recorded drug and allergy histories have resulted in more complete drug and allergy history information. Some of the results obtained from these interviews are summarized: patients taking prescription drugs at the time of admission-75%, patients taking prescription drugs during the past year-90%, patients taking non-prescription drugs within a few weeks of

Major Findings (cont'd)

admission-92%, and patients reporting a history of allergy to drugs, food, animals, clothing, tape, and iodine-31%. These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacist awareness of patient care. All medications brought to the hospital by the patient are obtained at time of the interview, and are stored in the pharmacy during the hospitalized period. The pharmacists are responsible for placing all known allergic reaction information on the front of the patients drug Kardex, and on the front of the patient chart. At the time of complication of the interview, the original is placed in the history section of the patients hospital chart and a duplicate is attached to the patients drug profile in the pharmacy. If the pharmacists feel that some of the information should be brought to the physicians attention immediately, he also communicates verbally to the physicians. These interviews have demonstrated that the pharmacist is interested in patient care, and that he can provide an additional useful service. The drug and allergy interview by the pharmacist has become an integral part of the clinical pharmacy program.

Significance to Biomedical Research:

More complete histories of medication usage are provided which may result in improved care of patients.

Proposed Course:

The present study will be continued.

Honors and Awards: None

Publications: None

Serial No: NCI-6237
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of a Patient Care Pharmacy Service

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove, William A. Cornelis, and Robert De Christoforo

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1-1/3
Professional: 1-1/3
Other: 0

Project Description:

Objective:

The pharmacist has traditionally been a product oriented person with little input to the actual care of the patient. The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team activity contributing to the patient's care.

Methods Employed:

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the patient care. The pharmacist attends morning and evening ward rounds, reviews charts, interviews patients and is familiar with the patient condition therefore he can intelligently discuss drug and therapy problems with the patient's physician. With this information, he can also provide the staff with drug information.

An integral part of this service is the drug surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile. The pharmacist receives a direct copy of the physician drug order and transcribes it to the patient drug profile. The drug profile includes the patient name, primary and secondary problems, physician name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocal drug

Methods Employed (con't)

dosages then reviews the drug profile against the new order for drug interaction, laboratory problem, dietary problem, possible adverse drug reactions. The drugs are then dispensed thru a unit dose system which provide accurate records. All returned doses to the pharmacy are investigated. The monitoring system involves evaluating the necessity of continued medication orders.

Major Findings:

The pharmacist has proven that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the health care team and in contributing directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test adnormalities, detecting possible drug induced diseases, providing drug information, counselling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs and reduced cost.

Significance to Biomedical Research:

A higher quality of drug prescribing and reduced medication problem has contributed toward better patient care and has enhanced the quality of the research data.

Proposed Course:

Present studies will be continued and additional methods to improve patient care, such as unit dose drug distribution, will be further developed.

Honors and Awards: None

Publications:

Grove, W.R., and Fortner, C.L.: Drug Therapy Surveillance by a Pharmacist in a Cancer Research Center: Hospital Pharmacy; Vol. 8; No. 2; 46-52; (Feb) 1973

Christian, D.G., Cornelis, W.A., and Fortner, C.L.: The Acute Leukemias: Journal of the American Pharmaceutical Association:NS12;9;473-481; (Sept)1972

Cornelis, W.A., Christian, D.G., and Fortner, C.L.: Hodgkin's Disease: Journal of the American Pharmaceutical Association:NS13;3;147-154; (Mar) 1973

Publications (Con't)

Fortner, C.L., SChimpff, S.C., Grove, W.R., and Greene, W.H.; Hyperalimentation As A Predisposing Factor To Bacteremia In Patients With Cancer; (Abst); Clinical Society and Commissioned Officers Assoc. of USPHS (accepted)

Serial No: NCI-6238
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of a Unit Dose Service and the Preparation of Parenteral Drugs by a Patient Care Pharmacy Service

Previous Serial Number: Same and 6269

Principal Investigator: Clarence L. Fortner

Other Investigators: William R. Grove, William A. Cornelis, and Robert De Christoforo

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 3-1/2
Professional: 2
Other: 1-1/2

Project Description:

Objectives:

To improving patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

Methods Employed:

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labelled with a name, strength, and controlled number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. All of these parenteral products are prepared aseptically in a laminar air flow environment, and are dispensed in a clearly labelled ready to use form. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particular matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patients name. This information is also recorded by drug and lot number for each investigational agent.

Major Findings:

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduces the possibility of contamination of the drug, permits nurses' to use time more effectively in direct patient care and in general allow the hospital to demonstrate adequate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record.

During the past year the pharmacy service has dispensed approximately 197,648 oral doses of medication to inpatients, 26,426 I.V. admixtures, and 4,139 doses of parenteral investigational cancer chemotherapeutic agents.

Significance to Biomedical Research:

By reducing medication errors, and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients has increased.

Honors and Awards: None

Publications: None

Serial No. NCI 6023
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radiation Therapy Service

Principal Investigator: Roger Byhardt,M.D.

Other Investigator: Kirkland C. Brace,M.D.

Cooperating Units: Baltimore Cancer Research Center and the U.S. Public Health Service Hospital, Baltimore, Maryland

Man Years: (July 1, 1972 - June 30, 1973)

Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

The Baltimore Cancer Research Center Program is centered on a group of protocols involving mainly primary tumors of the brain and acute myelocytic leukemia. The patients in both of these major programs receive radiation as an interval part of a combined therapeutic regimen. Patients with other types of tumors are treated for control of the primary disease or control of the pain-producing metastases. The general hospital has an active head and neck tumor service which requires both pre and post-operative irradiation as part of the treatment. Research activities constitute the vast majority of the patients treated in this department.

Methods Employed:

All modes of radiation therapy are available here for the treatment of all types of malignancies. The equipments consists of a 100 Kv superficial therapy machine, a 285 Kv orthovoltage machine, a 5000 curie Cobalt tele-therapy unit and a 6 Mev linear accelerator as soon as a treatment room is constructed. A supply of Cesium, Radium and Strontium sources are available for interstitial and intracavitary radiation therapy.

Major Findings:

The patient load increased substantially during the past year because of increased admissions on the Tumor Service. During the year ending March 31, 1973, 284 patients were seen in consultation. A total of 261 patients were

treated; the Baltimore Cancer Research Center supplied 187 patients and the the balance were from the general hospital. The breakdown of the treatments follow (April 1, 1972 - March 31, 1973):

	Tumor Service	Hospital Service
Superficial Therapy Treatments	11	65
Orthovoltage Therapy Treatments	19	30
Cobalt Therapy Treatments	2972	976
Intracavitary or Interstitial Radium & Cesium Applications	0	4
Strontium-90 Applications	0	4
P-32 Injections	3	0

Significance:

The progressive decline in the general hospital population has reduced the number of patients treated for the U.S. Public Health Service Hospital. The Baltimore Cancer Research Center increased activity has more than made up for this decline. The Hodgkin's disease protocol has shown increased activity over the past year.

Course:

The Radiation Therapy Service will continue to provide treatment for cancer patients both on the Research Service and for the general hospital. The closing of the hospital will not significantly reduce the patient load in this Department. The major problem still consists in the procuring of trained personnel and in our inability to install a linear accelerator which has already been purchased.

Honors and Awards:

None

Publications:

None

Serial No. NCI 6207
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radiological Physics - A Special Service Which is Supplied for Research Purposes

Principal Investigator: Kirkland C. Brace,M.D.

Other Investigators: Joan M. Zarriello,R.N. & Patricia K. Alemi

Man Years: (July 1, 1972 through June 30, 1973)

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

Objectives:

To apply the principles of Radiological Physics to the treatment of patients.

To provide non-patient service irradiations.

To provide a Radiation Safety Program for the Pharmacology Laboratories and Radiotherapy Department.

Methods Employed:

The three Radiation Therapy machines are calibrated periodically. Treatment plans are devised as needed.

Cells and animals were irradiated for investigators of the Pharmacology Laboratory during the past year. A total of 79 exposures from the Cobalt machine were provided. Irradiation services have also been provided to the Johns Hopkins Laboratory of Biology on 20 occasions this past year.

A Radiation Safety Program is maintained for the Pharmacology Laboratories in which radionuclides are kept or used. These laboratories are surveyed periodically for radionuclide contamination. The Radiation Therapy facilities (Cobalt-60 Teletherapy unit and various sealed sources of Radium, Cesium-137, and Strontium-90) are surveyed according to AEC Regulations.

Major Findings:

There has been no detectable leakage of any Radiotherapy sources, nor any significant amount of contamination in any Pharmacology Laboratory.

Proposed Course:

This institution does not have a Radiation Physicist and as a result there have been lapses in the radiation safety coverage. It may be necessary to hire an outside Physicist to carry on this program.

Honors & Awards:

None

Publications:

None

Serial No: NCI 6270
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Combination Chemotherapy and Radiotherapy in the Treatment of Hodgkin's Disease

Principal Investigator: Kirkland C. Brace,M.D.

Other Investigators: Roger Byhardt,M.D. and Peter H. Wiernik, M.D.

Cooperating Unit: Medicine Service, Baltimore Cancer Research Center

Man Years (July 1, 1972 - June 30, 1973)

Total: 1-1/4

Professional: 1/2

Others: 3/4

Project Description:

Objective:

The role of radiation therapy in the permanent control of early staged Hodgkin's disease is well-established. The use of chemotherapy in combination in the treatment of the advanced disease is now very clear. In this study we have combined these two modalities in hopes that it will be possible to provide permanent control of all but the most advanced disease. The initial study in this protocol was made with Stage I and Stage II Hodgkin's disease. Untreated patients were randomized in to three treatment groups. One group received conventional radiation therapy to the tumor-bearing lymph nodes and then adjacent uninvolved lymph node areas. A second group were treated with three courses of combination chemotherapy (MOPP) and this was followed by the irradiation of involved lymph node areas. The third group received initial irradiation to the involved lymph nodes followed by three courses of combination chemotherapy.

Methods Employed:

Three groups of Stage I and II Hodgkin's disease were obtained by randomization of newly diagnosed cases referred to this institution. One group received conventional radiation therapy to the involved areas and adjacent lymph node-bearing areas. A second group of patients were treated with three courses of

a combination chemotherapy MOPP and this was followed by irradiation of the involved lymph-node bearing areas. The third group of patients received irradiation to the involved lymph node areas followed by three courses of combination chemotherapy.

Major Findings:

A total of twenty-one patients randomized into this study have now been followed for not less than three years. There have been recurrences in the first two groups but as of this date there have been none in those patients that were treated with radiation therapy followed by chemotherapy.

Significance:

A statistical analysis of this initial data clearly reveals that the combination of radiation followed by chemotherapy reduces the incidence of recurrent disease in these patients. The odds of recurrence after three years is very low and the five year results are going to show that the combination provides superior control of disease to either modality alone.

Course:

This finding has stimulated a change in protocol and admissions have been extended to include all untreated Hodgkin's disease except III-B and IV. The group which received chemotherapy before irradiation has been eliminated and the current study will include only the radiation group and the radiation followed by six courses of chemotherapy. The initial analysis of our data sustains our impression that the combination is going to be superior to the radiation alone. In three years we should have a statistically significant answer.

Honors and Awards:

None

Publications:

" Combination Radiotherapy and Chemotherapy in the Treatment of Early Hodgkin's Disease", by Peter Wiernik, M.D. and Kirkland C. Brace,M.D. To be published in: Journal of Oncology

Serial No: 6294
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Total Body Irradiation in the Treatment of Advanced Lymphosarcoma

Principal Investigator: Kirkland C. Brace,M.D.

Cooperating Units: Baltimore Cancer Research Center, Medicine Service

Man Years: None

Project Description:

Objectives:

The study of the use of whole body irradiation in the treatment of lymphosarcoma, Stages III and IV has been discontinued.

SUMMARY REPORT

LABORATORY OF PHARMACOLOGY

In continuing studies on the clinical pharmacology of cancer chemotherapeutic agents, the human pharmacokinetics of adriamycin were elucidated and compared to previous data on daunorubicin. Adriamycin is extensively metabolized in the human but the pattern of metabolism differs somewhat from that of daunorubicin. From animal and human bile, conjugated metabolites of adriamycin and daunorubicin have been described for the first time. Since the urinary excretion of adriamycin is low in the human (@5% over 5 days), and studies in animals and human autopsy samples show a high biliary excretion, the biliary route was proposed as the major excretory route in the human. The application of these basic observations in patients led to the conclusions that increased toxicity is seen with both daunorubicin and adriamycin therapy in patients with hepatic dysfunction. These, in turn, have led to dosage regimen modifications for lower toxicity and increased therapeutic efficacy. The in vivo distribution and metabolism of the anthracyclines has shown that the parent drugs and glycoside metabolites are the major tissue components whereas aglycones account for small portions of the tissue drug. Physiological hypoxia stimulates the tissue glycosidase responsible for the metabolism of adriamycin and daunorubicin to their doexyaglycone derivatives. In studies on cellular control mechanisms, a macromolecular hepatic stimulator substance was partially purified. This material is present in the livers of weanling rats and can stimulate nucleic acid synthesis and cell division in vivo in rat liver 2- to 3-fold.

The development of a general assay utilizing the presence of polyamines in the urine of patients with diagnosed cancer has been described. These substances shown to be present in the urine of cancer patients at considerably higher concentrations than in the urine of non-cancer patients may prove to be of inestimable value, not only in detecting early cancer, but modulating chemotherapy in response to the patient's diseased state. At present, studies attempting to relate these polyamines to the amount of tumor tissue present are under active investigation. Difficulties relating to the sensitivity as well as the rapidity with which the polyamines can be determined have been decreased through use of the amino acid analyzer. This instrument provides great capabilities for rapid and accurate determinations of polyamines. The physiological role of the polyamines has been examined in a number of systems. The polyamine, spermidine, has been found to affect profoundly the metabolism of RNA. Not only is the synthesis of the substance altered, but the degradation as well. The Enzymology Section has also been interested in studying the sequence of an RNA isolated from a murine leukemia virus. Our experience to date has shown that the RNA can be isolated as two large macromolecules with a sedimentation constant of 30S. The 5' termini of both has been shown by us to be 5' uridylic acid.

Immunologic research has concentrated in in vitro systems designed to detect both host response to specific antigens as well as the amplification of responses to tumor specific antigens. The effects of double-stranded synthetic polyribonucleotides, polyadenylic and polyuridylic acid has been shown to play a unique role in amplifying responsiveness to antigens in vitro which are intrinsically weak or are at concentrations which preclude the demonstration of such responsiveness. In addition, it has been demonstrated that poly AU was capable of amplifying responsiveness to the measles virus, an agent which has not been

shown to elicit consistent responsiveness in vitro in those individuals known to be sensitive. The amplification effect of this agent in vitro and its known effectiveness in vivo is now being actively studied in terms of specific tumor immunotherapy. In addition, research has been carried out defining the role of complement in mediating tumor cell lysis both in vivo and in vitro. It has been observed that a close homology must exist between tumor-specific antibody and complement in order for tumor destruction to take place both in vivo and in vitro. When heterologous antiserum is utilized, such homology may not exist between the passively administered antibody and host complement. The reproducibility of the ability to produce tumor-specific antibody in the xenogeneic host utilizing blocking antibody was again demonstrated in a species other than the burro (rabbit). Immunotherapeutic studies were carried out in the AKR mouse designed to test the effectiveness of BCG in altering the incidence and progression of spontaneous leukemia in this strain. It was demonstrated that survival time was significantly increased in the BCG-treated groups. Despite significant prolongation of survival, all animals eventually developed leukemia. This latter work provides additional evidence that adjuvant immunotherapy may have a positive effect on the host with the predilection to develop tumor as well as on the host with tumor. Under the regimen utilized, however, cure was not achieved. Continued study of immune complex disease has indicated that subclinical immune complex deposition is ubiquitous among laboratory mice. We have suggested that immune complex deposition occurs during the natural history of a majority or all antibody responses in vivo. The kidney has become a focal point for studies in which minimal host immune responsiveness may occur in vivo. Continued studies in patients with neoplasia indicate that 7.5% (23/305) of those studied had subclinical immune complex deposition. The highest percentage of kidneys with immune complexes occurred in those from patients with acute leukemia (16% - 13/84) and the lowest (1.5% - 2/127) in those from patients with solid tumors.

Serial No. NCI-6012
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biohcemical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6012

Principal Investigator: Nicholas R. Bachur

Other Investigators: Ronald Felsted
Shigeru Takanashi
Malcolm Gee
Marta Kaber

Cooperating Units: Laboratory of Chemistry, National Heart & Lung Institute
Drug Development Branch, NCI, NIH

Man Years (July 1, 1972 to June 30, 1973)

Total:	4.4
Professional:	2.4
Other:	2.0

Project Description:

Objectives:

To study the biochemical pharmacology of selected cancer chemotherapeutic agents to provide a scientific foundation for their usage. To resolve the metabolic pathways of cancer chemotherapeutic agents and to study the interactions of these drugs and their metabolites with tissue components in vitro and with organized physiological systems in vivo.

Major Findings:

While investigating the metabolic conversions of adriamycin and daunorubicin in vivo in the rat, we observed that the bulk of these drugs were excreted in the bile. Chromatographic analysis of the bile from daunorubicin treated animals revealed that daunorubicinol was the major excretory product and accounted for about 70% of biliary drug excretion. In addition, a substantial portion of the drug was excreted as conjugated drug. Several of the biliary conjugates were isolated, purified, and analyzed. By using laboratory-purified substrate specific hydrolases and acid hydrolysis, we found that the rat bile conjugates were principally glucuronides of daunorubicin, daunorubicinol,

daunorubicinol aglycone, deoxydaunorubicin aglycone, and deoxydaunorubicinol aglycone.

Adriamycin excretion in the rat is quite different from daunorubicin since the parent drug is the major excretory product in the bile. Although about 25% of the drug in the bile is in more polar metabolites the structures of these has not been resolved.

Ours are the first reports of conjugated metabolites of the anthracycline drugs (also see NCI-6241). In addition, these studies indicate that the metabolism of these compounds is much more complex than previously imagined. Preliminary observations indicate that some of the conjugated metabolites may have cytotoxic activities.

Further progress in the isolation, purification, and characterization of daunorubicin reductase has been made. The enzyme has been purified to homogeneity from rat liver and rabbit liver. Similar preparations are being made from human tissue sources. Other drugs can interact with the purified daunorubicin reductase both as substrate and as inhibitors. The significance of these interactions are being assessed.

Significance to Biomedical Research and Program of the Institute:

The understanding of the biochemical alterations that active chemotherapeutic agents undergo is central to the understanding of their usage. This is of importance with the anthracycline antibiotics since we have observed a rather extensive metabolism of both adiamycin and daunorubicin *in vivo* and *in vitro*. Although these drugs have been used for several years, their metabolism has not been described.

Proposed Course:

We will isolate and purify the metabolites of both adriamycin and daunorubicin so they can be identified. Since we can also test them for cytotoxicity at a molecular level, at a cellular level and at a whole animal level, we will be able to obtain information concerning the toxicity of the specific metabolites as well as an overall understanding of their structure-activity relationships.

The mammalian enzymes which utilize adriamycin and daunorubicin as substrate will be purified and studied with the idea of utilizing them as control points to affect the efficacy of anthracycline therapy.

Honors and Awards: None

Publications:

Cradock, J.C., Egorin, M.J., and Bachur, N.R.: Daunorubicin Biliary Excretion and Metabolism in the Rat. Arch. Int. Pharmacodyn. 202: 48-61, 1973.

Serial No. NCI-6014
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Cellular Control Mechanisms Affecting Cell Growth and Differentiation

Previous Serial Number: NCI-6014

Principal Investigators: Nicholas R. Bachur
Douglas R. LaBrecque
Joseph Fleisher

Cooperating Units: None

Man Years (July 1, 1972 to June 30, 1973)

Total:	2.2
Professional:	2.2
Other:	0

Project Description:

Objectives:

To study the control mechanisms which affect cellular growth and differentiation. These studies are aimed at elucidating control principles effecting all levels of cellular machinery as well as various levels of physiological control of the organized functioning animal. To determine difference in controls between differentiated and nondifferentiated tissues and between malignant and normal tissues. To study the effects of control changes on physiologic processes.

Major Findings:

Hepatic Stimulator Substance was shown to stimulate the incorporation of ^{3}H thymidine into hepatic deoxyribonucleic acid of 34% hepatectomized rats. This stimulation was approximately 2.5 fold. Extracts from weanling rat livers contain the highest level of the stimulator substance but it does not become demonstrable until 10 to 12 hours post hepatectomy. In the weanling rats, diurnal rhythm of stimulator substance is observed with a peak activity at 3 to 9 A.M. and nadir at 6 to 9 P.M. A lag period of 10 hours exists between the injection of stimulator substance and the stimulation of DNA synthesis. Partial characterization of stimulator substance shows that it is a soluble material not sedimented by centrifugation for 2 hours at 140,000 xg. The

stimulator substance activity is stable to heating at 65°C or 100°C for 15 minutes but is lost when treated with 10% perchloric acid. By using molecular seives we have estimated the molecular weight of the stimulator substance to be approximately 10,000 - 15,000.

In our investigations of altered intermediary metabolism by malignant tissues we have attempted to quantify brain tumor mass by specific intermediates in the cerebral spinal fluid. Through the use of gas-liquid chromatography interfaced with computer analysis we have developed ultrasensitive methods for the analysis of cerebrospinal fluid. These techniques are being applied to our patient material at present.

Significance to Biomedical Research and Program of the Institute:

The isolation of materials from both plant and mammalian sources which effect the growth and differentiation of normal lymphocytes and hepatocytes is paramount in the study of the processes which control cellular activities. By using these purified materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways is possible, we are approaching techniques where chemotherapeutic agents may be used to control cellular growth, differentiation, and proliferation.

In that malignant growth may have altered metabolic pathways, we feel that ultrasensitive techniques may allow us to quantify tumor growth by measuring alterations in metabolite levels.

Proposed Course:

We intend to continue our studies on the identification and mechanism of action of the mammalian promotor substance. Also we will continue investigations on the analysis of human cerebral spinal fluid with relation to brain malignancy.

Honors and Awards: None

Publications: None

Serial No. NCI-6016
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pharmacodynamics of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6016

Principal Investigator: Nicholas R. Bachur

Other Investigators: M. Egorin
R. Hildebrand

Man Years (July 1, 1972 to June 30, 1973)

Total:	0.7
Professional:	0.2
Other:	0.5

Project Description:

Objectives:

To investigate the biochemical and physiological effects of anticancer drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Major Findings:

Several recent reports have implicated aglycone formation from administered anthracycline antibiotics as being causal to cardiotoxicity. Although aglycones are metabolites of the anthracyclines, we have not observed high levels of these materials in animal hearts. To assess the levels of aglycones in an animal subject to acute cardiotoxicity, the hamster, we studied acute changes in metabolite levels in these animals.

Intravenously administered daunorubicin (25 mg/kg) or adriamycin (5 mg/kg) are rapidly taken up and metabolized in tissues of the Golden Syrian hamster. In heart, lung, and kidney, the major metabolite of daunorubicin is daunorubicinol, whereas in liver daunorubicinol and deoxydaunorubicinol aglycone are prominent metabolites. Adriamycin is metabolized to a lesser degree than daunorubicin in the hamster, but several metabolites are observed. As with daunorubicin, aglycone metabolites of adriamycin are low except in the liver. Tissue hypoxia may be a contributing factor for the metabolism of the anthracycline antibiotics to aglycone metabolites, especially in liver and kidney. Levels of

daunorubicin fluorescence fall rapidly in all post-mortem tissues but changes in adriamycin fluorescence are small. Also the hamsters tolerate the large doses of daunorubicin (25 mg/kg) well, but are killed by similar doses of adriamycin. These studies indicate physiological and biochemical differences in these two structurally similar drugs.

Significance to Biomedical Research and Program of the Institute:

Although both adriamycin and daunorubicin are active anticancer agents, both agents produce cardiotoxicity in patients after prolonged high level dosage. We have attempted to delineate the metabolic pathwasy of these drugs and possibly to relate the metabolism to the cardiotoxicity. There is no clear evidence to date that such a relationship exists.

Proposed Course:

Through the continued investigation of the pharmacodynamics of these drugs, we may be able to determine why the drugs possess such differences in the therapeutic index and spectrum of action.

Honors and Awards: None

Publications: None

Serial No. NCI-6241
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Clinical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6241

Principal Investigator: Nicholas R. Bachur

Other Investigators: Robert S. Benjamin
Shigeru Takanashi
Peter H. Wiernik

Cooperating Units: Medicine Service, Baltimore Cancer Research Center, NCI

Man Years (July 1, 1972 to June 30, 1973)

Total:	1.4
Professional:	1.4
Other:	1

Project Description:

Objectives:

To study the disposition of cancer chemotherapeutic agents in humans with respect to absorption, distribution, biotransformation, and excretion. To characterize drug effects, potency, and maximum efficacy. To evaluate the toxicity of cancer chemotherapeutic agents in man and observe various aspects of biological variations and other factors such as age, body weight, sex, route of administration, time of administration, rate of inactivation and excretion, tolerance, pathological state, genetic factors, and drug-drug interaction. To devise appropriate dosage schedules. To develop ancillary therapy to minimize toxic effects of drugs without effecting the therapeutic value.

Major Findings:

Since we have observed that the biliary excretion of both daunorubicin and adriamycin play a major role in their disposition, we have attempted to estimate the pattern of biliary excretion in the human. We have investigated the human biliary excretion of these drugs in human bile samples obtained on autopsy. Bile from patients treated with daunorubicin (60 mg/M^2 x 3) or adriamycin (80 mg/M^2) for acute nonlymphocytic leukemia or solid tumors was extracted,

chromatographed on thin layer chromatograms and the components were quantified by fluorometry. Bile from daunorubicin treated patients contained mostly daunorubicinol @75%. Other fluorescent components were as follows: daunorubicin @10%, conjugates @10% and aglycones @5%. In adriamycin bile the components were adriamycin @50%, conjugates @30%, less polar metabolites @8%, and aglycones @12%. Metabolites were identified through chromatography, spectral analysis, mass spectroscopy, and enzymatic degradation. Aglycones found were daunorubicin aglycone, deoxydaunorubicin aglycone, and deoxydaunorubicinol aglycone. The human conjugates were similar to what we found in the rat. Glucuronide conjugates of daunorubicin and deoxydaunorubicin aglycone have been identified.

Significance to Biomedical Research and Program of the Institute:

In the light of our findings that patients with hepatic dysfunction had increased toxicity following adriamycin treatment these findings have increased significance. Most importantly, we have been able to correlate hepatic dysfunction with increased plasma levels of adriamycin and adriamycin metabolites.

Proposed Course:

We will continue to study the metabolism and pharmacokinetics of cancer chemotherapeutic agents and attempt to relate these to the clinical observations.

Honors and Awards: None

Publications:

Greene, W.H., Huffman, D.H., Schimpff, S., and Bachur, N.R.: Single High Dose Daunorubicin Therapy for Acute Non-Lymphocytic Leukemia. Cancer. 30: 1418-1427, 1972.

Serial No. NCI-6210
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: The Development of Specific RNAses as an Aid in the Sequencing of tRNAs from Murine Leukemia Viruses

Principal Investigator: C. C. Levy

Other Investigator : M. Schmukler

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	: 1/2
Professional	: 1/2
Other	: 0

Project Description:

Objectives
The isolation from microbial sources of nucleases which will hydrolyze long chain nucleic acids at specific base linkages as an aide in the sequential analysis of RNA from various murine leukemia viruses.

Major Findings
The technique of enrichment culture was employed to isolate a number of microorganisms from soil which could degrade RNA at specific nucleotide bonds. Thus far nucleases specific for bonds containing cytidylic acid and uridylic acid have been obtained from two of the organisms. A third, Serratia sp., elaborates an enzyme which shows preferential specificity for adenylic acid linkages. The second of the two organisms described, Citrobacter sp., which hydrolyzes uridylic acid internucleotide bonds, has shown interesting alterations in its specificity under the influence of the polyamine, spermidine. The Citrobacter nuclease shows no activity towards polycytidylic acid until spermidine is introduced into the reaction mixture. At this point the degradation of cytidylic acid containing nucleotide bonds is rapid and complete. Of considerable interest also is that this is the first instance in which spermidine has been shown to have a direct effect on an enzyme. Of all the polyamines tested, the enzyme binds spermidine preferentially.

Significance to Cancer Research
Nucleic acid metabolism, or more correctly the alteration of nucleic acid metabolism, is believed to play an important role in cancer induction. It is hoped that an understanding of the sequential relationship of one nucleic acid to the other in the construction of a large chain polymer in tumor-

causing viruses may provide a clue as to the nature of cancer induction. In addition, the effect of spermidine on the enzyme which hydrolyzes polyuridylic acid is of considerable interest. The reason is that in the urine of patients with diagnosed cancer there appears to be an increase in polyamine levels. The relationship of spermidine to the Citrobacter enzyme may have significance in terms of the physiological changes which are occurring in the cell as the tumor progresses.

Proposed Course

The projected course of this study is to utilize these enzymes as a means of sequencing RNA isolated from tumor viruses. It is hoped that by establishing partial sequences in a number of RNA's from various tumor viruses, some cancerous relationship between RNA's will be seen.

Publications

Levy, C. C., Mitch, W. E. and Schmukler, M. Effect of polyamines on a ribonuclease which hydrolyzes RNA at uridylic acid residues. J. Biol. Chem., in press.

Levy, C. C., Mitch, W. E. and Schmukler, M. Influence of polyamines on the hydrolysis of polynucleotides by Citrobacter nuclease. In Polyamines in Normal and Neoplastic Growth (Ed. D. H. Russell) Raven Press, New York, in press.

Marotta, C. A., Levy, C. C., Weissman, S. M. and Varricchio, F. The specificity and use of a ribonuclease from Enterobacter sp. in the sequence analysis of B. stearothermophilus 5S RNA. Biochemistry, in press.

Schmukler, M., Friedling, S. P. and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. II. Studies with synthetic polyribonucleotides. Biochim. Biophys. Acta 268, 403-410, 1972.

Friedling, S. P., Schmukler, M. and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. I. Purification and general properties. Biochim. Biophys. Acta 268, 391-402, 1972.

Serial No. NCI-6242
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Polyamines and Cancer: Functional Significance

Principal Investigator: D. H. Russell

Other Investigators : C. C. Levy

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	:	1
Professional	:	1
Other	:	0

Project Description:

Objectives
To define the functions of putrescine, spermidine, and spermine, and to use this information for cancer control.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in tissues by dansylation of amines, separation by thin layer chromatography, and fluorometric detection.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine and from ^{14}C-spermidine formation from ^{14}C-putrescine; or from ^{14}C-spermine formation from ^{14}C-spermidine.
4. Routine determination of the relationships between RNA content and polyamine concentrations.

Major Findings
1. Utilization of the various methods for detecting polyamines in the urine of cancer patients continues to reaffirm the validity of the initial observation; namely, that these compounds are elevated in patients with cancer. A new amino acid analyzer technique allows us to evaluate polyamine levels in the serum of cancer patients.
2. Considerable data have been accumulated concerning the effects of polyamines on nucleolar RNA polymerase activity. Nucleolar RNA polymerase (RNA polymerase I) was partially purified from rat liver nucleolar preparations and stabilized by the addition of albumin. It was observed that there was a

2-3 fold increment in RNA polymerase I activity when the enzyme was assayed in the presence of magnesium. In addition to the stimulation of RNA polymerase I activity, the polyamines caused structural alterations in the nucleolus. Electron micrographs showed definitive changes in the nucleoli when the nuclei were isolated in the presence of 2.5-10 mM putrescine, spermidine, or spermine, in contrast to that seen with similar concentrations of magnesium. Nuclei isolated by the addition of either spermidine or spermine as the stabilizing cation had nucleoli that were significantly larger than those isolated with magnesium. In general, when the nuclei were isolated in the presence of magnesium, the nucleoli were compact and electron dense, whereas in the presence of spermidine or spermine, the nucleolonemas were less aggregated, and the nucleoli contained lacunae. Both spermidine and spermine (10 mM) caused the formation of ring-shaped nucleoli. Further studies indicate differential effects of polyamines on RNA polymerase I and II.

Significance to Cancer Research

Considerable progress has been made in understanding the functional role that polyamines play in the growth process. The possibility that these compounds may be enhanced, directly or otherwise, in processes leading to abnormal growth is beginning to be realized. Thus, for example, the structural alterations in cellular organelles (cited above) is suggestive that other modifications of structure leading to abnormal growth can occur.

Proposed Course

1. Further studies involving regular screening of sarcoma, carcinoma, and ANLL patients before, during and after chemotherapy will be undertaken to determine if polyamine levels at any point in therapy can serve as a reliable indicator for future chemotherapy.
2. Anticancer agents will be examined for their ability to inhibit tumor growth in various animal systems.
3. Since spermidine affects RNA so profoundly, further studies on biosynthetic enzymes will be continued, to determine if these enzymes can be used as indicators of neoplastic growth.

Publications

Russell, D. H.: Increased polyamine concentrations in the urine of human cancer patients. Nature 233: 144-145, 1971.

Russell, D. H., Levy, C. C., Schimpff, S. C. and Hawk, I. A.: Urinary polyamines in cancer patients. Cancer Res. 31: 1555-1558, 1971.

Russell, D. H., Levy, C. C. and Taylor, R. L.: Increased DNA associated with rat liver nucleoli when isolated with spermidine. Biochem. Biophys. Res. Commun. 47: 212-215, 1972.

Russell, D. H.: Polyamines in growth - normal and neoplastic. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 1-13.

Marton, L. J., Graziano, K. D., Mardiney, Michael R., Jr. and Russell, D. H.: Specific increases in polyamines in mixed lymphocyte reactions. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 215-219.

Gfeller, E., Levy, C. C. and Russell, D. H.: Structural and biochemical changes in the nucleolus in response to polyamines. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, in press.

Marton, L. J., Vaughn, J. G., Hawk, I. A., Levy, C. C. and Russell, D. H.: Elevated polyamine levels in serum and urine of cancer patients: Detection by a rapid automated technique utilizing an amino acid analyzer. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, in press.

Serial No. NCI-6245
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Studies on Human Platelet Carbohydrate Metabolism: (1) The Effect of Cyclic AMP: (2) Interactions of Thrombin and Nucleotides and Their Effects on Glycolysis; (3) Effects of Storage and Various Drugs on Glycogenesis and Glycogenolysis

Principal Investigators: P. Zieve
M. Schmukler

Other Investigators : None

Cooperating Unit : Division of Hematology, Baltimore City Hospitals

Man Years : (July 1, 1972, through June 30, 1973)

Total	1
Professional	1/2
Other	1/2

Project Description:

Objectives
To study the metabolism of platelets in order to define the biochemical factors involved in platelet aggregation.

Major Findings
1. ATP and ADP, which inhibit thrombin-induced stimulation of lactogenesis, interact with thrombin in such a way that its mobility in an acrylamide gel electrophoresis system is markedly increased. The change in electrophoretic mobility is not due to an alteration of the molecular weight of thrombin since this is unchanged. Possibly, the nucleotides combine with or alter the charge of the thrombin. It is this process which may be responsible for the ability of the nucleotides to inhibit thrombin effects on platelet lactogenesis.
2. The platelet has been shown to incorporate glucosamine and mannose into protein. Incorporation, which is linear for about 60 minutes, is optimal at a substrate concentration of 4 mM. Incorporation is greater in the whole cell than in a disrupted system. Thrombin is able to cleave off incorporated label, suggesting that at least some of the glycoprotein made gets into the platelet membrane.

Significance to Cancer Research
Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often

controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course

Studies of glucosamine and mannose incorporation will be continued. Subcellular locations of incorporation will be delineated and the significance of this synthesis to platelet aggregation will be studied.

Publications

Schmukler, M. and Zieve, P. D. The effect of glucose feeding on fatty acid synthesis in human platelets. J. Lab. Clin. Med. 80: 231, 1972.

Zieve, P. D. and Schmukler, M. The interaction of thrombin and nucleotides: Effects on glycolysis in human platelets. Biochim. Biophys. Acta, 1973 (in press).

Serial No. NCI-6247
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Purification of Bacterial and Mammalian Nucleases with Differing Residue Specificities

Principal Investigator: M. Schmukler

Other Investigators : C. C. Levy

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	:	1/2
Professional	:	1/4
Other	:	1/4

Project Description:

Objectives
Purification of a ribonuclease from Klebsiella species, and to delineate the characteristics and properties of human ribonucleases and determine whether any cancer-related changes occur. To purify ribonucleases from plasma and white cells in normal subjects and patients with cancer, and characterize and compare their properties.

Major Findings
1. Klebsiella nuclease which was purified 550-fold, is inhibited by a number of synthetic polyribonucleotides, including poly G, poly I, poly CG, poly GI, and poly GU. In addition, native and denatured DNA, and the multistranded polymer, poly A·poly U, are also inhibitors. Some evidence was obtained showing that some of these compounds are able to inhibit other ribonucleases and these compounds may be useful in studying the mechanisms of action of ribonucleases.
2. A ribonuclease purified 2000-fold from human plasma was found to have a high specificity for cleavage at cytidylic acid residues producing either 2'-3' CMP or oligonucleotides ending in cytidylic acid. A search for this enzyme in human tissues has thus far been unsuccessful.

Significance for Cancer Research
The study of enzymes concerned with nucleic acid metabolism is essential to an understanding of the mechanisms of carcinogenesis and cancer control since there is obviously an alteration in nucleic acid metabolism in cancer and since most chemotherapeutic agents are directed in some way against nucleic acid metabolism. Nucleases which have some residue specificity should be valuable tools in the delineation of nucleic acid structure. It is also pos-

sible that nucleases with some type of specificity may be useful as chemotherapeutic agents. The studies with the polyribonucleotide inhibitors of nuclease activity may be useful in suggesting techniques whereby specific functions of malignant cells could be disrupted.
2. Nucleic acid metabolism is altered in malignancy. Study of enzymes involved in nucleic acid metabolism may offer clues as to the nature of the malignant process. These enzymes, also, may be indicators of certain types of malignancies.

Proposed Course

The specificity of the human ribonuclease will be further studied. A search will be made for its tissue of origin. When the tissue of origin is found, its ribonucleases and nucleic acids will be studied. The enzyme is reportedly elevated in the plasma of patients with multiple myeloma. This observation will be checked and an attempt will be made to obtain myeloma cells for study.

Serial No. NCI-6287
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1972

Project Title: Studies on Glycosidases of Human Platelets

Principal Investigators : M. Schmukler
P. D. Zieve

Other Investigators : None

Cooperating Unit : Division of Hematology, Baltimore City Hospitals

Man Years : (July 1, 1972, through June 30, 1973)

Total	: 1 1/4
Professional	: 3/4
Other	: 1/2

Project Description:

Objectives
To study platelet glycosidases, their release reaction, and the relationship of these enzymes to platelet function and stability.

Major Findings
Platelet glycosidases can be released by action of thrombin. Such thrombin-induced release is inhibited by PGE, ATP, CTP, and GTP. Release is decreased after storage, but addition of glucose to the incubation medium augments release in both fresh and stored platelets. Concanavalin A causes release of αmannosidase but not of the other glycosidases. On the other hand, Concanavalin A blocks thrombin-induced release of the other glycosidases. The data suggest that αmannosidase has a different subcellular location from the other glycosidase and/or is released by a different mechanism.

Significance to Cancer Research
Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course
An attempt will be made to find substances which will stabilize platelet function during storage. Effects of chemotherapeutic agents on glycosidase release will also be investigated. An attempt will be made to distinguish αmannosidase membrane release receptors from those responsible for release of other glycosidases.

Publications

Schmukler, M. and Zieve, P. D. Studies of glycosidases in fresh and stored platelets. J. Lab. Clin. Med. 80: 675, 1972.

Serial No. NCI-6288
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Polyamine Biosynthesis in Rapidly Growing Tissues

Principal Investigator: D. H. Russell

Other Investigators : None

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	:	1
Professional	:	1
Other	:	0

Project Description:

Objectives
To elucidate the pattern of synthesis and accumulation of polyamines in rapidly growing biological systems.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in tissues by homogenization in acid, alkaline butanol extraction and high voltage electrophoresis for separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine and from ^{14}C-spermidine formation from ^{14}C-putrescine; or from ^{14}C-spermine formation from ^{14}C-spermidine.
4. Routine determination of the relationships between RNA content and polyamine concentrations.

Major Findings
In mammary gland there is a very close correlation between polyamines and RNA content. In Xenopus, injection of putrescine directly stimulates RNA synthesis.

Significance to Cancer Research
An attempt is being made to elucidate the control process in rapidly growing cells. An understanding of this process, it is hoped, will gain understanding of the neoplastic process itself.

Proposed Course

These studies will be continued with particular emphasis on elucidating in considerably greater detail the relationship in the nucleolus between RNA and polyamine synthesis.

Publications

Russell, D. H. and McVicker, T. A.: Polyamine metabolism in mouse liver after partial hepatectomy. Biochim. Biophys. Acta 244: 85-93, 1971.

Russell, D. H. and McVicker, T. A.: Polyamines in the developing rat and in supportive tissues. Biochim. Biophys. Acta 259: 247-258, 1972.

Russell, D. H., Shiverick, K. T., Hamrell, B. B. and Alpert, N. R.: Polyamine synthesis during initial phases of stress-induced cardiac hypertrophy. Amer. J. Physiol. 221: 1287-1291, 1971.

Russell, D. H.: Drug stimulation of putrescine and spermidine syntheses. Biochem. Pharmacol. 20: 3481-3491, 1971.

Gfeller, E. and Russell, D. H.: Autoradiographic distributions of ^{3}H-putrescine and ^{3}H-uridine in a Xenopus liver cell line. Z. Zellforsch 120: 321-331, 1971.

Russell, D. H. and McVicker, T. A.: Polyamine biogenesis in the rat mammary gland during pregnancy and lactation. Biochem. J. 130: 71-76, 1972.

Manen, C. A. and Russell, D. H.: Polyamines in marine invertebrates. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 277-288.

Wylie, C. C. and Russell, D. H.: The stimulation of RNA synthesis in mature amphibian oocytes by the micro-injection of putrescine. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, in press.

Serial No. NCI-6289
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Purification of S-adenosyl-L-methionine Decarboxylase from Rat Liver and from other Biological Sources

Principal Investigator: D. H. Russell

Other Investigators : C. C. Levy

Man Years : (July 1, 1972, through June 30, 1973)

Total	:	1/4
Professional	:	1/4
Other	:	0

Project Description:

Objectives

1. To determine if there are separate decarboxylases for spermidine and spermine synthesis.
2. To determine if this system for polyamine biosynthesis is the same when isolated from widely divergent sources.

Methods Employed

1. S-adenosyl-L-methionine decarboxylase activity determined by $^{14}CO_2$ released from S-adenosyl-L-methionine when either putrescine or spermidine is used as a substrate. When putrescine is used as the substrate, there is spermidine formation. When spermidine is used as the substrate, there is spermine formation.
2. Sephadex gel filtration and fractionation.
3. DEAE-cellulose column chromatography.
4. Calcium hydroxylapatite column chromatography.
5. Affinity chromatography.
6. Protein determinations according to the method of Lowry et al.
7. Molecular weight determinations by the methods of Andrews and Martin and Ames.

Major Findings

S-adenosyl-L-methionine decarboxylase has been purified more than 350-fold from rat liver by a new procedure. It was found that 40% saturation with ammonium sulfate actually caused a loss of between 60-90% of enzyme activity. These results are in contrast to the effect of ammonium sulfate recently reported on the enzyme purified from ventral prostate. The molecular weight of the enzyme as determined by Sephadex gel filtration and by sucrose density centrifugation was found to be approximately 50,000. Enzyme activity was

inhibited by sulfhydryl group inhibitors and by inhibitors of pyridoxal phosphate systems. Spermidine could be substituted for putrescine at all stages of purificaiton, but there was always a 2:1 preference for putrescine. A similar type of enzyme has been isolated from sea urchins (L. pictus) and from L1210 cells grown in culture. The molecular weight of these enzymes ranges between 50,000-59,000, which seems very similar to that found for the liver system. The enzyme from sea urchins is inhibited by those substances affecting the rat liver system. At present electrophoresis is being used to determine if the enzyme will separate into subunits.

Significance to Cancer Research

Since the polyamine biosynthetic pathway is intimately tied with rapid growth, an understanding of the enzymes of the polyamine biosynthetic pathway would enable us to better elucidate inhibitors of the rapid growth process. With a purified enzyme system, one can test inhibitors that might be of value in cancer chemotherapy.

Proposed Course

To examine in detail if the enzyme isolated from L1210 cells differs in any way from other biological material. And to determine if these differences can be used as a means of detecting cancerous systems.

Publications

Feldman, M. J., Levy, C. C. and Russell, D. H.: The purification of S-adenosyl-L-methionine decarboxylase from rat liver: Inability to separate decarboxylase from spermidine synthesis. Biochem. Biophys. Res. Commun. 44: 675-681, 1971.

Feldman, M. J., Levy, C. C. and Russell, D. H.: Purification and characterization of S-adenosyl-L-methionine decarboxylase from rat liver. Biochemistry 11: 671-677, 1971.

Serial No. NCI-6290
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Development of a Sensitive Method for Polyamine Determination Utilizing Gas Liquid Chromatography

Principal Investigator : D. H. Russell

Other Investigators : L. J. Marton
C. C. Levy

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	: 1/4
Professional	: 1/4
Other	: 0

Project Description:

Objectives
To develop a rapid and sensitive method for putrescine, spermidine and spermine quantification utilizing gas liquid chromatography.

Methods Employed
1. Assays were performed with a Packard Model 7400 gas liquid chromatography with hydrogen flame ionization detectors. Various packing materials for 4-ft coiled glass columns with a 2 mm internal diameter were used. It was determined that 3% OV-17 on 100-120 mesh Gas Chrom Q (Applied Science Laboratories) was the ideal packing material for optimal separation of derivatized polyamines. For good separation the polyamines had to be derivatized by reaction with trifluoroacetic anhydride.

Major Findings
A rapid and sensitive method for polyamine determination was developed using gas liquid chromatography. Accurate determinations of nanogram quantities of putrescine, spermidine and spermine were made after an assay time of approximately 15 minutes. As an internal standard for the gas liquid chromatography, 1-7 diaminoheptane was found to be ideal. This compound has a retention time between putrescine and spermidine so that by using the internal standard at the beginning of the extraction, the recovery rate of each polyamine could be determined.

Significance to Cancer Research
This method can be used to assay polyamine levels in cerebral spinal fluid, in urine, or in blood. Since the method is rapid and extremely sensitive,

large scale screening for polyamines in various body fluids can be undertaken. Thus, the development of this method should serve to validate previous findings of polyamines as indicators of neoplasia.

Proposed Course

The development of a sensitive amino acid analyzer technique has led to the discontinuance of this project. Also, this method proved to be nonquantitative.

Project terminated.

Serial No. NCI-6291
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1972

Project Title: Polyamine Metabolism in Spleens and Livers of Leukemic Mice. Effects of Potential Anticancer Agents

Principal Investigator: O. Heby

Other Investigators : D. H. Russell

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	:	1
Professional	:	1
Other	:	0

Project Description:

Objectives
To define the functions of putrescine, spermidine, and spermine, and to understand possible control mechanisms of their synthesis in leukemic mice.

Methods Employed
1. Spectrofluorometric quantitation of putrescine, spermidine, and spermine in tissues after dansylation and thin layer chromatography separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine- and spermine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine (carboxyl-^{14}C).

Major Findings
A study conducted during the first months as a Visiting Fellow indicates that the ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities are elevated in spleens of leukemic mice 24 hours after intraperitoneal L_{1210} leukemia cell inoculation. The ornithine decarboxylase activity decreased by day 5 and was normal on day 8 of tumor growth, whereas the S-adenosyl-L-methionine decarboxylase activity remained elevated during the first eight days of tumor growth. The maximal survival of leukemic mice was nine days. It was shown that 5-azacytidine markedly suppressed the enzyme activities in leukemic mice when injected daily (day 1-9). The drug injections were started one day after tumor transplantation, in order to certify 100% tumor "takes." Except for the effects on the enzymes, the drug treatment caused a 300% increase in life span of the host mice. At the time

when the drug treatment was interrupted, ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities increased rapidly both in drug-treated leukemic mice and in drug-treated control mice, indicating that 5-azacytidine exerts a general suppression on polyamine-synthesizing enzymes.

Significance to Cancer Research

An understanding of the early events that occur upon an invasion of malignant cells is necessary in order to structure effective chemotherapy.

Proposed Course

1. Continued experiments to confirm the results obtained.
2. Since it has been shown (Hofer and Hofer, Cancer Res. 31, 402, 1971) that implantation of L_{1210} intraperitoneally is followed by rapid migration of tumor cells from the injection site to other organs, it would be important to study the ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities in spleens and livers of mice every 2-3 hours following the inoculation of L_{1210} leukemia cells. L_{1210} metastasis is very high during early phases (first 24 hours) of tumor growth, and much slower in advanced tumors, and it might be possible to correlate early changes of enzyme activities in spleen and liver with the reported invasion of tumor cells in these organs.
3. Regular screening of polyamine synthesis and accumulation before, during, and after chemotherapy by various potent agents, to determine if polyamine levels at any given point in therapy can serve as a reliable indicator for future chemotherapy, i.e. best possible dosage schedule, when to stop, when to continue chemotherapy.
4. It would be useful to obtain data on the cellular composition of the spleen during tumor growth and chemotherapy, to understand what causes the marked weight changes observed, and in order to, if possible, distinguish between enzymatic changes in metastatic leukemic cells and other kinds of cells.

Publications

Russell, D. H. Effects of methotrexate and cytosine arabinoside on polyamine metabolism in a mouse L_{1210} leukemia. Cancer Res. 32: 2459-2462, 1972.

Heby, O. and Russell, D. H. Depression of polyamine synthesis in L_{1210} leukemic mice during treatment with a potent antileukemic agent, 5-azacytidine. Cancer Res. 33: 159-165, 1973.

Heby, O. and Russell, D. H.: Changes in polyamine metabolism in tumor cells and host tissues during tumor growth and after treatment with various anticancer agents. In Russell, D. H. (ed.): Polyamines in normal and neoplastic growth. New York, Raven Press, 1973, pp. 221-237.

Serial No. NCI-6902
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: The Characterization of Viral RNA Isolated from the AKR Murine Leukemia Virus
Previous Serial Number : NONE
Principal Investigator : C. C. Levy

Other Investigators : J. J. Frank

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total	: 1/2
Professional	: 1/2
Other	: 0

Project Description:

Objectives
To isolate from an AKR murine leukemia virus its RNA in undegraded form so that the 5' and 3' terminii of the RNA can be elucidated. The specific objective is to determine the polarity at which a message contained within the RNA is read on the mouse ribosome.

Major Findings
Thus far, the isolation of undegraded viral RNA has proved of considerable difficulty. A technique which has given some success is one in which the virus is disrupted by a combination of phenol, sodium dodecal and salt solution. The viral RNA is extracted into the aqueous phase and then subjected to gel electrophoresis. After the electrophoresis, the viral RNA is removed from the gel and subjected to alkaline phosphatase treatment. The phosphate at the 3' and 5' terminii are removed and the RNA at the 5' end phosphorylated with polynucleotide kinase and ATP^{32}. The 5' terminus of the RNA is then released by a suitable phosphodiesterase to give 5' phosphate nucleotides. Analysis by ion exchange chromatography and paper electrophoresis has shown that uridylic acid is the nucleotide at the 5' end of the RNA. Studies are currently in progress to use other enzymes to determine the 3' terminus as well. The viral RNA has also been hydrolyzed with base specific ribonucleases. Preliminary studies have shown that one enzyme can degrade viral RNA into oligonucleotides with chain links approximately 10-20 nucleotides in length.

Significance to Cancer Research
An understanding of the primary sequence of the viral RNA should be helpful in the elucidation of the mechanism by which the virus may replicate itself. Thus

if that portion of the viral RNA responsible for the enzyme "reverse transcriptase" is known, then it may be possible to design drugs which can destroy it.

Proposed Course

The projected course is to continue the analytical studies and to establish the polarity by which the RNA is read on the mouse ribosome.

Serial No. NCI-6903
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: Development of Specific RNAses as an Aid in the Sequencing of DNA from Various Viral Tumors
Previous Serial Number : NONE
Principal Investigator : C. C. Levy

Other Investigators : D. A. Cook

Cooperating Units : None

Man Years : (July 1, 1972, through June 30, 1973)

Total : 1/2
Professional : 1/2
Other : 0

Project Description:

Objectives
The isolation from microbial sources of a DNAse which will hydrolyze deoxyribonucleic acid at a specific base linkage as an aid in the sequential analysis of DNA obtained from a tumor virus.

Major Findings
The technique of enrichment culture was employed to isolate DNAses from a microorganism which degrades DNA at specific nucleotide linkages. The organism has been classified as a Flavobacter and the enzyme has been characterized to some degree. The enzyme has a molecular weight of at least 200,000 and presumably breaks up into four, perhaps six, subunits. The enzyme appears to be an endonuclease in that alteration of the terminii of various substrates has no effect on enzyme activity. The enzyme has strong specificity for deoxyadenylic acid, since it will not attack ribonucleic acid or polyribonucleotides under any conditions. The enzyme should be helpful in elucidating the primary sequence of DNA from tumor viruses.

Significance to Cancer Research
The ability to degrade deoxyribonucleic acid at specific linkages when the nucleic acid is obtained from viral sources should be of intense interest in sequencing the DNA of a tumor virus. It is hoped to compare regions of the DNA with comparable areas isolated from RNA tumor viruses.

Proposed Course
To isolate a homogeneous DNA from viral sources and to attempt a sequential analysis of the nucleotide composition.

Serial No.: NCI-6020

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanisms of Human Lymphocyte Reactivity to Non-Self

Previous Serial Number: SAME

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Kenneth D. Graziano, Ph.D.
Carl C. Levy, Ph.D.
Morton Schmukler, M.D.

Cooperating Units: Section of Enzymology and Drug Metabolism

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

1. To enhance the sensitivity of immunologic induced blastogenesis in vitro.
2. To study reactivity to viral determinants using the mumps virus as a model.
3. To study the role of the human granulocyte as an antigen in the blastogenic response.

Methods Employed:

Short-termed tissue cultures in which the proliferative response of cells in question were monitored by measuring the incorporation of tritiated thymidine.

Major Findings:

1. The effects of double stranded synthetic polyribonucleotides, poly adenylic and polyuridylic acid (poly AU), on the tritiated thymidine incorporation of cultured leukocytes was studied. Amplification of responses to suboptimal concentrations of strong antigenic stimuli (purified protein derivative, tetanus toxoid, and allogeneic cells) was con-

Major Findings (continued)

1. sistently observed. In addition, poly AU amplified the tritiated thymidine incorporation of sensitized leukocytes responding to a "weak" stimulus (measles virus). In contrast to the results with cellular or soluble antigenic stimuli, poly AU depressed phytohemagglutinin-induced reactivity. Studies with purified lymphocytes suggest that poly AU-induced amplification requires the presence of a radio-resistant surface-adherent cell population.
2. Mumps virus inactivated with betapropriolactone was found to be a potent stimulus in initiating lymphocyte immune responsiveness. Response was found to be independent on antigen concentration, and a radio-resistant, adherent cell population. Measurable lymphocyte transformation after primary exposure to this virus was found to persist for up to 40 years, and to correlate well with delayed hypersensitivity.
3. Granulocytes were found not to act as an antigen in the mixed lymphocyte reaction.

Relevance:

1. Amplification of this in vitro assay of cell-associated immune function should increase its sensitivity and have a wide range of usefulness in the further experimental and clinical study of responses to weak and suboptimal cellular and soluble antigenic stimuli. This statement is especially relevant in studies designed to test for sensitivity to tumor specific antigens.
2. The demonstration of lymphocyte tritiated thymidine incorporation in response to mumps virus demonstrates that this assay may be a useful method to study the course of cellular reactivity after viral exposure as well as a means to detect previous exposure to other viruses.
3. The inert role played by the granulocyte in the mixed lymphocyte reaction as well as its possible inhibitory role played at high concentrations suggests that methods allowing for removal of this cell from this reaction system should be utilized.

Proposed Course:

To evaluate the basic mechanisms relating to the initiating of this amplification and to take advantage of this phenomena in studying the cellular responsiveness of host to tumor.

Honors and Awards: None

Presentations:

Colloquium of the C.N.R.S., Paris, France, 1972.

Publications:

Chess, L., Levy, C.C., Schmukler, M., Smith, K. and Mardiney, M.R., Jr.: The Effect of Synthetic Polynucleotides on Immunologically Induced Tritiated Thymidine Incorporation: Amplification of Response. Transplantation 14: 748-755, 1972.

Mardiney, M.R., Jr., Chess, L., Levy, C.C., Schmukler, M., and Smith, K.: The Use of Double Stranded Synthetic Polynucleotides Amplifying Immunologically Induced Lymphocyte Proliferation. Papers of the Colloquium of the C.N.R.S. on "The Investigation and Stimulation of Immunity in Cancer Patients", Paris, France, Editor - Georges Mathe, 1972.

Smith, K., Chess, L. and Mardiney, M.R., Jr.: The Characteristics of Lymphocyte Tritiated Thymidine Incorporation in Response to Mumps Virus. Cellular Immunology 5:597-603, 1972.

Mardiney, M.R., Jr., Bock, G.N. and Chess, :: The Role of the Human Granulocyte as an Antigen in the Mixed Lymphocyte Reaction: The Lack of Stimulation. Transplantation 14:274-278, 1972.

Serial No.: NCI-6248

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: To Determine the Effect of Freezing on the Transformation of Lymphocytes in the Mixed Lymphocyte Reaction and in Response to Specific Antigens and PHA.

Previous Serial Number: SAME

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Kenneth D. Graziano, Ph.D.
Keith Kalmbach

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/8
Other:	1/8

Project Description:

Objectives:

Previous work has defined that lymphocyte immunologically induced tritiated thymidine incorporation could be evaluated in a manner which abrogates many of the variables which usually preclude quantitative comparisons of lymphocyte responsiveness in vitro on a day to day basis. The technique which utilizes the simultaneous culture of frozen-stored lymphocytes obtained at various periods of time has been demonstrated to allow for quantitatively identical responses in lymphocytes from the normal host to allogeneic cells, PHA, and specific antigens (responses to a specific antigen being identical in the absence of re-stimulation between lymphocyte sampling). It order to improve the ability for investigations in this area, an attempt was made in improving some of the technical aspects of freezing lymphocytes.

Methods Employed:

1. Slow freezing of lymphocytes in media containing 10% DMSO.
2. Short-term tissue cultures in which the proliferative response of the cells in question were evaluated by measuring the incorporation of thymidine.

Major Findings:

A freezing controller was developed which is capable of establishing an electronic analog of the desired cooling slope, provides continuous temperature measurement over the specimen to be cooled, and adds vaporized liquid nitrogen as required to keep specimen temperature alligned with cooling slope analog. Several advantages are a natural result of this approach:

1. System continually compensates for changes in its specific heat through 3 phases as well as variability of biological materials.
2. Once freezing run is started, no additional attention is required, i.e., manual over-ride or specimen/medium gradient change.
3. No trial and error was needed to establish the required specimen temperature gradient.
4. System senses for precise mement of liberation of heat of fusion and provides sufficient coolant to keep specimen in line with desired cooling curve eliminating temperature undershoot.
5. Desired cooling rate (0.1° C/min to 10° C/min) is maintained down to a preset lower limit (0° C to minus 100° C).

Application:

This controller will allow for studies and optimum freezing characteristics of all types of mammalian cells. In addition, its use will decrease the need for technical observation during the freezing period.

Honors and Awards: None

Presentations: None

Publications:

Kalmbach, K., and Mardiney, M.R., Jr.: An Improved System for Controlled Rate Cooling of Biological Materials. Cryobiology, 1972.

Chess, L., Bock, G.N., and Mardiney, M.R., Jr.: Restoration of Reactivity of Frozen-Stored Human Lymphocytes in the Mixed Lymphocyte Reaction and in Response to Specific Antigen. Transplantation 14:728-733, 1972.

Serial No.: NCI-6249

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients

Previous Serial Number: SAME

Principal Investigator: John C. Sutherland, M.D.

Other Investigators: Michael R. Mardiney, Jr., M.D.
Roy Van Markham, Jr.
Eugene F. Cimino
Walter P. Drake

Cooperating Units: Department of Pathology, USPHS Hospital, Baltimore, Maryland.

Man Years:

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

1. To determine the association of long-term viral disease and neoplasia in man.
2. To evaluate the extent of immune complex deposition in the normal laboratory mouse.

Methods Employed:

Localization of immune complex deposition was determined by fluorescent microscopy. Putative suggestion of immune complex deposition was dependent on the finding of both IgG and $Beta_{1C}$ globulin in a segmental pattern in glomerular basement membrane. In the human model, kidneys for study were obtained both postmortem and by open renal biopsy. The finding of complex deposition was then followed by a fluorescent screening assay utilizing various antisera directed against viral agents putatively involved in neoplasia. In the animal model, a study was conducted to determine the extent of subclinical immune-complex nephritis among 12 strains of normal

Methods Employed (continued):

(132 animals) and 2 strains of germ-free (25 animals) mice.

Major Findings:

1. Human Study:

Pursuant to our report of last year, we now wish to state that further studies have confirmed the specificity of staining of 2 kidneys from patients with acute myelocytic leukemia with antiserum against feline leukemia virus (FeLV). These studies include the absence of positive staining after absorbing the FeLV antiserum with ether-treated FeLV and with ether treated murine leukemia virus (1 kidney) and the identification by complement fixation of solubilized oncornaviral antigen (both kidneys) and antibody (1 kidney) and by gel diffusion of oncornaviral antibody (1 kidney) in whole kidney homogenates. These studies do not implicate FeLV as an etiologic agent in human neoplasia as only the gs-3 or interspecies antigen of the mammalian oncornaviruses was identified in these kidneys. However, they do suggest that human leukemia is homologous to feline and murine leukemias in its etiology.

Renal tissue has been obtained by open biopsy during staging operations for Hodgkin's disease as well as from autopsies to compare the occurrence of immune complexes in antemortem material as compared to postmortem material. Immune complexes were found in 23 percent (5/22) of kidneys sampled during staging laparotomies in patients with Hodgkin's disease and in 9 percent (2/23) of kidneys taken at autopsy from patients with Hodgkin's disease. There was no clinical or histologic evidence of glomerulonephritis in these patients and kidney and we refer to these complexes as subclinical. Although the antigens in these complexes have not yet been identified, we can make 2 general statements concerning the patients in whom glomerular-entrapped complexes were found postmortem. First, these patients did not exhibit either clinical of histological evidence of terminal viral infections. Second, no immune complexes were found in the kidneys of 4 patients who had either clinical or histologic evidence of terminal viral infection.

A total of 304 kidneys from autopsy have been examined for the presence of subclinical immune complexes. They have been found in 7.5 percent (23/305). The highest percentage of kidneys with immune complexes occurred in those from patients with acute leukemia (16%-13/84) and the lowest (1.5%-2/127) in those from patients with solid tumors. In 34 kidneys from non-cancer patients, immune complexes have been found in 15% (5/34). In addition to immune complexes, gamma globulin alone has been found in another 11 kidneys (1 acute leukemia, 1 chronic leukemia, 1 lymphoma, 4 solid tumors and 4 without cancer). $Beta_{1C}$ globulin (C3) was found alone in yet another 10 (3 acute leukemias, 4 lymphomas, 1 solid tumor, and 1 without cancer).

2. Mouse Model:

Major Findings (continued):

Mouse $Beta_{1C}$, IgG, IgM, and IgA were detected in renal glomeruli by immunofluorescence. Glomerular deposition of IgM occurred in 95.4%, IgG occurred in 85.6%, IgA in 62.2% and $Beta_{1C}$ in 78.7% of normal mice in a distribution typical of immune complex disease. Guinea pig $Beta_{1C}$ fixed in vitro to the glomeruli deposits can be removed by acid treatment. Germ-free mice showed a lower incidence in deposits than normal mice. The capillary-linear pattern of fluorescence, characteristic for planted or fixed antigen was noted in 5.1% (8/127) of the animals and in 80% (4/5) of the retired breeder SJL/J mice examined. Minor proteinuria and glomerular changes were noted with no correlation with glomerular deposits and no systemic disease was detected.

Relevance:

1. Human Model:

The occurrence of subclinical immune complexes deposited in the kidneys of patients with a variety of diseases affords another parameter for evaluating host response to both known and unknown antigens. Knowledge concerning the exact significance of these deposits awaits the identification of the antigen involved. The identification of these or yet unknown antigens, however, may contribute significantly to knowledge concerning the pathogenesis of a number of diseases. This conclusion is supported directly by the previous identification of a mammalian oncornaviral antigen in 2 leukemic kidneys. It is also supported at least indirectly, by the Hodgkin's disease studies in which complexes were shown to be present at the time of staging laparotomies, early in the clinical course of the disease, and in postmortem material, by the lack of association of immune complexes with either clinically or histologically evident terminal viral infections.

2. Mouse Model:

The ubiquitous occurrence of complex disease in the normal mouse suggest that clearance by fixation to glomerular basement membrane may be a common route for the catabolism of a select population of antigen-antibody complexes due to the natural history of all or a majority of antibody responses in vivo.

Proposed Course:

1. To continue to screen for antigen in these complexes.
2. To continue to elute antibody from these kidneys and test for specificity.
3. To continue screening patients for this type of injury.

Honors and Awards: None

Presentations:

Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, 1973.

Publications:

Sutherland, J.C., and Mardiney, M.R., Jr.: Immune Complex Disease in the Kidneys of Lymphoma-Leukemia Patients: The Presence of an Oncornavirus Related Antigen. Journal of the National Cancer Institute, 1973.

Markham, R.V., Jr., Sutherland, J.C., and Mardiney, M.R., Jr.: The Ubiquitous Occurrence of Immune Complex Localization in the Renal Glomeruli of Normal Mice. Lab. Invest., 1973.

Markham, R.V., Jr., Sutherland, J.C., and Mardiney, M.R., Jr.: The Ubiquitous Occurrence of Immune Complex Localization in the Renal Glomeruli of Normal Mice. Abstract, Fed. Proc., 1973.

Sutherland, J.C., Markham, R.V., Jr., Ramsey, H.E., and Mardiney, M.R., Jr.: Subclinical Immune Complex Nephritis in Patients with Hodgkin's Disease, In Symposium on "Human Tumors Associated with Herpesviruses" edited by Dr. Paul Levine and Dr. John Moloney, 1973.

Serial No.: NCI-6274

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Production of Tumor Specific Antibody in a Xenogeneic Species Utilizing Blocking Antibody: The Characteristics of Reactivity with the Complement System.

Previous Serial Number: 6274 and 6019

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Walter P. Drake
Eugene F. Cimino

Cooperating Units: None

Man Years:

Total	2
Professional:	1
Other:	1

Project Description:

Objectives:

A broad study of the interaction of putatively anti-tumor antibody directed against EL4 cells was studied in relation to the effect of formalin fixation on the preservation of cellular antigenicity; the ability of such cells to be utilized as immunogens to produce tumor specific antibody; and the relationship of the complement system in enabling antisera to be specifically cytotoxic for the tumor cell both in vitro and in vivo.

Methods Employed:

Standard assays for the evaluation of antibody cytotoxic activity in vitro were utilized. These are complement dependent systems.

Major Findings:

1. Immunization of rabbits with formalin-fixed EL4 lymphoma cells of the C57BL/6 mouse was found to yield anti-tumor sera equivalent in cytotoxicity to that obtained with fresh viable cells.

Major findings (continued)

2. Formalin-fixation of passive antibody to tumor cells was effective in modifying subsequent harvested anti-tumor serum so that the antiserum had diminished reactivity to determinants against which passively administered antibody was directed.
3. It was found that a close homology between the complement source and the antibody utilized was necessary to generate antibody activity in vitro against the specific target cell. This homology was not necessarily in accord with the homology suggested by studies in which activity against red blood cells was evaluated.
4. The reproducibility of the ability to produce tumor specific antibody in the xenogeneic host utilizing blocking antibody was again demonstrated in a species other than the burro (rabbit).
5. The utilization of antisera produced in concentrated form (namely concentrated by fractionation and lyophilization) allowed for the development of cytotoxic activity 5-7 times that seen in the absence of lyophilization. The sera obtained by this method was more effective in tumor sera therapy than were unconcentaated sera.
5. Improvements in the cytotoxic assay were made utilizing formalin-fixed cell preparations as standards for use in an automated trypan blue assay.

Relevance:

The above work again defines that antibody production to cellular antigens can be altered by the simultaneous administration of homologous antibody to determinants common to tumor and normal cells. The use of the formalin technique has added additional parameters concerning the feasibility of collection and storage of cells in a state which has at least in this system, enabled the development of antibody similar in specificity as that developed by the use of fresh cells. The studies within the complement system has allowed us to re-evaluate previous studies performed both in this laboratory and elsewhere in which in vivo therapy using heterologous sera was not effective. In many of these experiments, homology between the antibody utilized and the complement mediating cytotoxicity was probably poor. Our conclusion at this time is that it is essential to evaluate the homology of the antitumor serum being utulized and the complement source mediating cytotoxicity of that anti-tumor serum in all future experiments in which in vivo cell kill effect of antibody is being evaluated.

Proposed Course:

1. To further utilize this approach to define tumor specific antigens on neoplastic cells.
2. To continue to evaluate various parameters of achieving specific cytotoxic activity against the tumor cell in vitro.
3. To extend this approach in defining tumor specific antigens on human neoplastic cells.

Honors and Awards: None

Presentations:

The American Association for Cancer Research, Atlantic City, New Jersey, 1973.

Publications:

Weiner, R.S., Hubbard, J.D., and Mardiney, M.R., Jr.: Production of Tumor Specific Antibody in the Xenogeneic Host: Use of Blocking Antibody. Journal of the National Cancer Institute 49:1063-1070, 1972.

Drake, W.P., Ungaro, P.C., and Mardiney, M.R., Jr.: Formalin-Fixed Cell Preparations for Use in the Automated Trypan Blue Cytotoxic Assay. Transplantation 14:127-130, 1972.

Ungaro, P.C., Drake, W.P., and Mardiney, M.R., Jr.: The Formalinization of Antibody to Tumor Cells in Altering the Immune Response. Cancer Res. 32:2241-2247, 1972.

Ungaro, P.C., Drake, W.P., and Mardiney, M.R., Jr.: Augmentation of Anti-tumor Activity by the Use of Lyophilization. Journal of the National Cancer Institute 50:201-204, 1973.

Serial No.: NCI-6292

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunologic Effects of BCG

Previous Serial Number: 6292

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Walter P. Drake

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

The present study was carried out to define the effect of the chronic administration of BCG into AKR mice as a both preventive and therapeutic measure to either inhibit or delay the onset of spontaneous leukemia in this strain. We felt this study of immune modulation on spontaneous leukemia in the AKR mouse was especially relevant since this animal has recently been shown to be immunologically responsive to the specific etiologic agent causing his disease.

Methods Employed:

Female AKR mice were randomly grouped at 8 weeks of age and treated every second week thereafter until 42 weeks of age with intraperitoneal injections of BCG or saline. 140 female AKR mice were randomly divided into groups of 20. Mice were injected with 0.1×10^6 to 5.4×10^6 viable BCG organisms.

Major Findings:

Animals given the highest dose of BCG suffered the greatest mortality in earliest stages of the experiment; 11 died before 21 weeks of age. These data agreed with data from parallel experiments and suggested that extremely high doses of BCG have an early adverse effect on the longevity of a good

Major Findings (continued)

proportion of AKR mice. A dose of 0.68 x 10^6 viable organisms per injection offered optimal survival. A marked break off point between this group and all the groups occurred at 38 weeks of age. Although the survival time was significantly increased in the BCG groups, all animals evaluated developed leukemia.

Relevance:

This work provides additional evidence that adjuvant immunotherapy may have a positive effect on the host with a predilection to develop tumor as well as on the host with tumor.

Proposed Course:

To further evaluate agents capable of enhancing host immunologic response.

Presentations: None

Publications:

Ungaro, P.C., Drake, W.P., and Mardiney, M.R.,Jr.: Repetitive Administration of Bacillus Calmette-Guerin in the Treatment of Spontaneous Leukemia of AKR Mice. Journal of the National Cancer Institute 50:125-128, 1973.

Mardiney, M.R., Jr., Chess, L., Bock, G.N., Ungaro, P.C., and Buchholz, D.H.: Immunologic Effects of BCG in Patients with Malignant Melanoma: Papers of the Colloquium of the C.N.R.S. on "The Investigation and Stimulation of Immunity in Cancer Patients", Paris, France, Editor - Georges Mathe, 1972.

Chess, L., Bock, G.N., Ungaro, P.C., Buchholz, D.H., and Mardiney, M.R., Jr.: Immunologic Effects of Bacillus Calmette-Guerin in Patients with Malignant Melanoma: Specific Evidence for Stimulation of the "Secondary" Response. Journal of the National Cancer Institute, August, 1973.

Serial No.: NCI-6250

1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ultrastructural and Tissue Culture Studies of Acute Leukemic Cells of Patients Before and During Treatment

Previous Serial Number: SAME

Principal Investigator: Frances T. Sanel

Other Investigators: Peter H. Wiernik, M.D.

Cooperating Units: Tumor Service

Man Years:

Total:	1/4
Professional:	1/8
Other:	5/8

Project Description:

Objectives:

Continuing studies are directed toward determining the responsiveness to and cytological aspects of phagocytosis-provoking agents such as thorium dioxide, and viable bacteria,on peripheral blood and bone marrow cells from patients with acute leukemia before and during treatment. Histochemical and cytochemical studies on this material as well as concommitant search for virus are continued.

Major Findings:

Aberrant cytomorphology and deficiency of lysosomal enzymes in leukemic cells have been observed. Myeloperoxidase deficient leukemic granulocytes from a patient with myelomonocytic leukemia were replaced by a population of cells with normal amounts of this enzyme after chemotherapy. Although the ability to phagocytose test material was not impaired in the untreated state, lysosomal breakdown of ingested material was not observed.

Massive aggregates of microfilaments in leukemic cells from untreated patients and their reappearance in relapse after dissappearance in remission have been noted in several cases.

Proposed Course:

1. Continued study of monocytic and myelomonocytic leukemias with phagocytosis provoking agents.
2. Cytochemical study of the glycocalyx of leukemic cells and utilization of freeze-etch techniques to elucidate the "zipperlike junctions" formed by some leukemic cells under test conditions.
3. Soft-agar tissue culture to induce differentiation of leukemic stem cells and responsiveness of these cells to chemotherapeutic agents.
4. Cytochemical study of microfilaments.

Scientific Exhibit:

Sanel, F.T. 1972. Zipper-like Junctions in Human Leukemic Cells. Washington Electron Microscopy Society, May Meeting.

Serial No.: NCI-6272

1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Myelomonocytic Leukemia in an Animal Model System: Electron Microscopy and Biological Experiments

Previous Serial Number: SAME

Principal Investigator: Frances T. Sanel

Other Investigators: None

Cooperating Units: Laboratory of Viral Diseases (Dr. Janet Hartley)
Microbiological Associates (Dr. J.C. Parker)

Man Years:

Total:	5/8
Professional:	1/8
Other:	1/4

Project Description:

Objectives:

To study etiology, passaging under varying conditions and trans-species grafting of transplantable, muramidase-producing myelomonocytic WEHI-3 tumor cells utilizing techniques of short term tissue culture, hematological, histological, histochemical and ultrastructural techniques.

Precise identification of the specific virus type within the murine oncornavirus group, and further evaluation of their oncogenic capability.

To determine the phagocytic competence of myelomonocytic neoplastic cells whose normal counterparts actively ingest particulates.

Major Findings:

1. Balb/c Mice:

Muramidase-producing tumor cells contained peroxidase positive granules additionally confirming their identity as non-lymphoid precursors.

We identified "C" type viral particles by electron microscopy in tumor cells

Major Findings (continued):

spleen, liver, and bone marrow. They were tested as tumor extracts by complement fixation and XC mouse embryo cytopathogenicity tests and they contained the group specific antigen (gs) of murine leukemia viruses and demonstrated high levels of infectivity. Recent results of cell-free passage into newborn Balb/c mice reveal massive production of "C" type viruses after a four month latency period. The disease in these animals differing from that in donor mice still remains to be characterized since the majority of mice treated with cell-free filtrates developed lymphatic leukemia, lymphoma, or hind leg paralysis and in a few instances, massive liposarcomas containing C particles, with no evidence of lymphomatous disease.

Phagocytosis experiments demonstrated tumor cells to be weakly responsive; however, in some cells phagocytosis occurred concomittantly with virus synthesis.

In several cell transplant generations, unusual rodlike structures were observed in tumor cells. These were continuous with the rough endoplasmic reticulum and occasionally were formed on the same cistern that demonstrated budding "A" type particles in another area. We speculate that these structures may be a cellular response to viral infection, may be a manifestation of neoplastic transformation, or that they may represent aberrant viral precursor elements.

2. Trans-Species Studies:

Trans-species passage of Balb/c tumor cells into newborn inbred Fisher rats resulted in induction of a transplantable lymphoid leukemia with massive thymomas, mesenteric tumors, enlarged spleens and systemic dissemination of leukemic cells. "C" type viral particles in tumor extracts examined by complement fixation and XC tests contained the gs antigen of MuLV and also demonstrated very high titers of infectivity.

Transfer of tumor cells from Fisher rats into newborn Balb/c mice induced lymphoid neoplasia in the latter.

Significance to Cancer Research:

These animal systems could be valuable models for the study of etiology, diagnosis and therapy of human neoplasia.

Proposed Course:

1. Continued study of the oncogenic potential of the "C" type particles.
2. Further study of the diseases in Balb/c mice and Fisher rats induced by cell-free filtrates of the WEHI-3 tumor.
3. Therapeutic studies on the WEHI-3 Balb/c animals in collaboration with Dr. M. Walker and Dr. P. Wiernik

Proposed Course (continued):

4. Freeze-etch studies of virus producing cells.
5. Soft-agar tissue culture of WEHI-3 tumor cells directed toward differentiation, virus production and responsiveness to chemotherapeutic agents.
6. Further study of the unusual cytoplasmic rodlets.

Publications:

Sanel, F.T. 1972. Unusual Intracytoplasmic Structures in Leukemic BALB/c Cells Producing Infectious "C" Type Particles. J. Cell Biol. 55:225a.

Sanel, F.T. 1973. Studies of Myelomonocytic Cells in Balb/c Mice Producing Infectious "C" Type Viruses. Cancer Res. 33:672.

ANNUAL REPORT

CANCER THERAPY EVALUATION BRANCH

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

Office of the Chief

Within the expanded scope of the Division of Cancer Treatment, the Cancer Therapy Evaluation Branch operates in two major functional areas:

1. Coordination and monitoring of all clinical trials performed with new anticancer drugs sponsored for clinical trial by the National Cancer Institute,

2. Development, coordination, and monitoring of combined modality approaches to cancer treatment.

As part of the expansion into these two functional areas, the Cancer Therapy Evaluation Branch will be restructured into two branches reporting to an Associate Director for Cancer Therapy Evaluation. These two will be:

1. Investigational Drug Branch, with responsibility for monitoring and coordinating clinical trials on new anticancer drugs sponsored by the Division, liaison with the Food and Drug Administration, and distribution of investigational drugs throughout the United States and the world.

2. Combined Modality Branch, with responsibility for developing extramural disease-oriented treatment programs with combined modality approaches and monitoring various disease-oriented treatment thrusts being sponsored in other parts of NCI or ongoing in international programs.

The Division of Cancer Treatment (DCT) includes within its expanded activities the drug development and clinical testing aspects of the former Chemotherapy Program. The new functions involve the improvement of cancer therapy results through the development and clinical testing of new combined modality approaches. These combined modality approaches will be developed mainly along disease-oriented lines, with the major emphasis being given to the solid tumors which constitute the bulk of cancer deaths in this country.

The main thrust in the combined modality area will be with chemotherapy plus surgery and/or radiotherapy and/or immunotherapy. This

is logical both from the standpoint of the long established DCT expertise in chemotherapy and from the fact that chemotherapy is the only modality of unquestioned effectiveness in killing tumor cells anywhere in the body. Chemotherapy, either with drugs alone or in combination, can cure some patients with at least eight different kinds of cancer.

The philosophic base of the combined modality approach is the recognition that both surgery and radiotherapy have plateaued in their ability to cure solid tumors. They are localized modalities which kill tumor cells only where they are applied, and technically it is not feasible to increase the scope of their application for the tumors in which they are effective. They fail to cure many patients, even when they remove all the tumor visible to the naked eye or diagnostic x-ray film. The reason for this failure is felt to be the presence of disseminated microscopic disease foci at the time of surgical excision of the primary tumor, which many times includes the surrounding tissue and part of the regional lymph nodes.

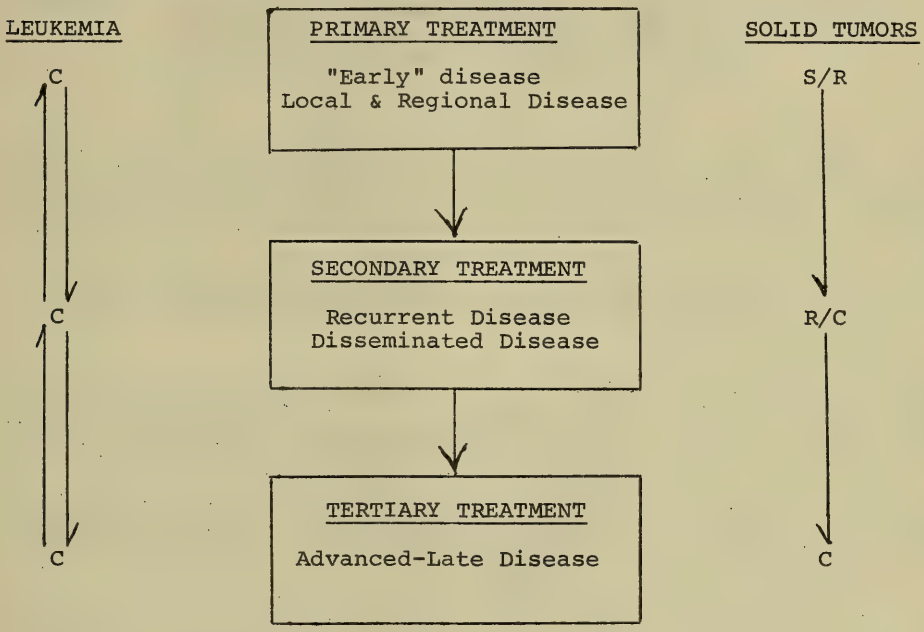
Chemotherapy, when used optimally, has the potential to eradicate these metastatic foci. The drug regimens which have shown the highest degree of activity in the advanced disease will be the prime candidates for use in the combined modality approach. The degree of cell kill necessary to shrink a bulky solid tumor mass by greater than 50% is quite large. If this degree of cell kill could be directed against the relatively small tumor burden remaining after surgical excision, perhaps eradication of the last neoplastic cell can be achieved.

Appendix A contains a preliminary review of an analysis being conducted to identifiy the solid tumors most likely to be responsive as well as the antitumor drugs of potential effectiveness in combined modality approaches.

The major successes of chemotherapy have been in the hematologic malignancies, especially acute lymphocytic leukemia and advanced Hodgkin's disease. These triumphs of cure and long disease-free survival have not been translated to the common solid tumors which are the major cause of cancer mortality in this country. Many reasons have been put forth for this disparity of results, among them the differing kinetics of the tumors and the relative accessibility of the tumor cells to significant drug concentrations. One additional factor, which is often neglected, is the point in the treatment strategy for a given disease at which chemotherapy is introduced.

As a general rule, the major potential for cure in any tumor is in the initial therapeutic approach. In leukemia, which is a disseminated disease, chemotherapy is the initial treatment. On the other hand, in solid tumors without disseminated disease surgery and/or radiotherapy are the primary approaches. The therapeutic flow in leukemia is chemotherapy at all stages of disease. The optimal chemotherapeutic regimens are used for primary treatment. New drugs and

regimens are tried in recurrent disease and the successful ones are integrated into the primary therapeutic regimens. In solid tumors, chemotherapy is relegated, almost exclusively, to secondary or tertiary treatment after surgery and/or radiotherapy fail and the disease is advanced and disseminated. The secondary or tertiary treatment is rarely curative in any tumor, including hematological malignancies. It is understandable, therefore, that the chemotherapy presently used in solid tumors is not curative although tumor regression, palliative benefit, and some survival increases are achieved. Any comparison of the results of chemotherapy in solid tumors and hematological malignancies should take into consideration these differences in treatment shown below:



C = Chemotherapy

R = Radiotherapy

S = Surgery

The proposed therapeutic strategy for increasing cure rates in solid

tumors involves integration of drugs into combined modality approaches for primary treatment according to the following scheme:

I. Test new drugs and combinations in advanced disease.

↓

II. Develop optimal chemotherapy regimen for primary treatment of disseminated disease.

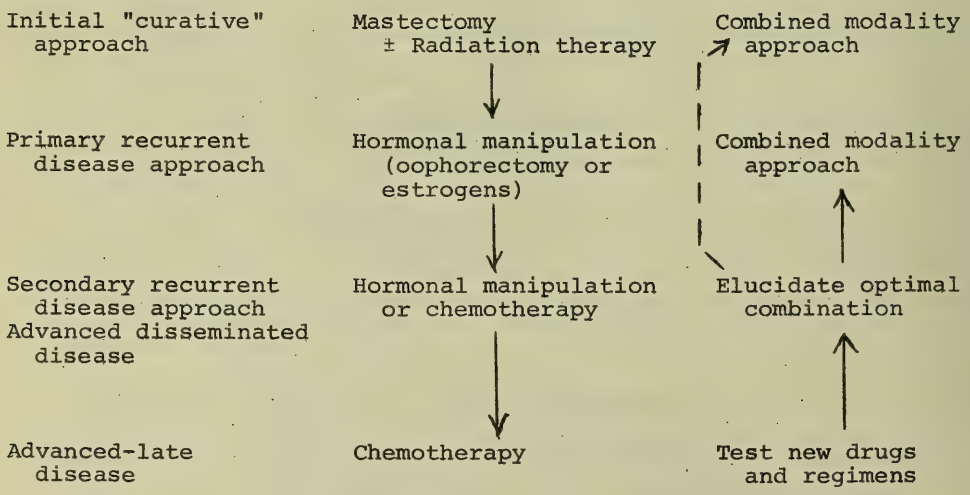
↓

III. Integrate optimal chemotherapy regimen into combined modality approach for primary treatment of local and regional disease.

In this scheme, new drugs and combinations would be tested in advanced disease and those showing positive results would move into the primary treatment of disseminated disease. The optimal regimen evolved in this situation would then be integrated into a combined modality approach for primary treatment of local and regional disease.

Such a proposal for a therapeutic attack against breast cancer is schematically outlined as follows:

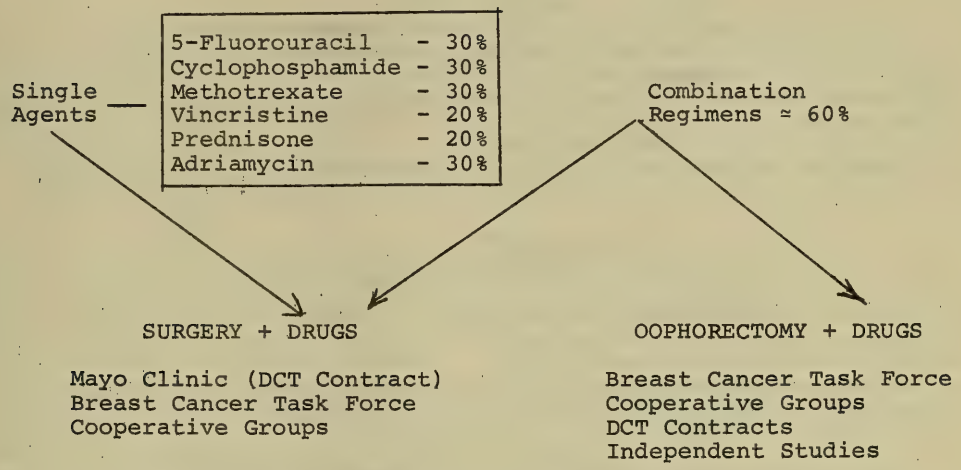
Treatment Flow



The solid arrows show the standard treatment flow for this disease, beginning with mastectomy and moving through hormonal manipulation

and eventually to chemotherapy. The broken arrows indicate the concerted efforts of the Division of Cancer Treatment and the Breast Cancer Task Force which might integrate chemotherapy into the earlier stages of treatment.

The specific chemotherapeutic approach being considered is shown below:



Six single agents exist which produce objective regression of tumor in advanced disease in 20 to 30 percent of the patients treated. Combination regimens involving these six drugs can achieve objective regression in approximately 60% of the patients. Protocols are being developed to test these combinations with oophorectomy for the treatment of primary recurrent disease. Additional protocols are underway or being developed for single agents and combinations in surgical adjuvant studies for the initial curative attack.

The Cancer Therapy Evaluation Branch of the Division of Cancer Treatment is now analyzing therapeutic results and planning future studies in the manner outlined above. Regimens in advanced disease will be analyzed not only for response rate, palliative value, and survival gain, but also for their potential use in earlier treatment stages. Disease-oriented protocols will be approached from both the point of view of the questions they ask and their integration into the total therapeutic flow for the specific tumor. The format of data reporting in new studies will be improved so that each study adds to the total knowledge of the tumor in question, as well as answering the specific questions posed in the protocol.

Currently, the Division of Cancer Treatment has 11 disease-oriented combined modality approaches to cancer treatment in progress or in the active planning stage:

1. Lung Cancer Working Party
2. Gastrointestinal Tumor Study Group
3. Breast Cancer Service
4. Brain Tumor Study Group
5. Testicular Tumor Study Group
6. Osteosarcoma Study Group
7. Ovarian Tumor Program
8. Pediatric Tumor Program
9. Malignant Lymphoma Program
10. Sarcoma Program
11. Acute Leukemia Program

The Cancer Therapy Evaluation Branch has undertaken the organization of two of these groups.

The first is the Gastrointestinal Tumor Study Group which will be supported under contract and includes the following institutions:

1. Mayo Foundation
2. Roswell Park Memorial Institute
3. U.C.L.A.
4. Harvard University
5. Yale University

The philosophy of organization in this group is the belief that disease-oriented combined modality groups should:

1. Contain a small number of highly selected institutions with expertise in oncology in general and the specific tumor in particular, and

2. Have a member from each modality from every institution.

The Gastrointestinal Tumor Study Group chose adenocarcinoma of the pancreas for its initial attack. The group recognized the disturbing rise in incidence of this tumor which now makes it the fourth leading cause of cancer deaths in the United States for both sexes. The dismal results with standard therapy make new therapeutic thrusts imperative and require establishment of mechanisms for continued study of the history of the tumor under treatment.

The group has developed two protocols through the following sequential mechanism evolved by the members:

I. Modality oriented committees meet and decide on recommendations.

II. Committees report to entire group and a broad consensus is reached on protocol approach.

III. Small combined modality committee appointed

to write actual protocol draft.

IV. Protocol draft discussed and ammended in modality oriented committees.

V. Committees report to entire group and protocol finalized.

The first is a prospective, randomized, phase III study of 5-fluorouracil (5-FU) and supervoltage radiation therapy as an adjuvant in patients with potentially curable pancreatic adenocarcinoma of the ductal, acinar, and undifferentiated varieties (Figure 1). The purpose of the study is to determine by controlled evaluation if postoperative treatment with 5-FU and supervoltage radiation can substantially improve disease-free interval and survival in this potentially curable group of patients.

The second study is a controlled evaluation of high dose (6000 r) radiation therapy, high dose radiation plus 5-FU, and medium dose (4000 r) radiation plus 5-FU in patients with localized but unresectable disease (Figure 2). Although radiation therapy at conventional (3000-4000 r) dosages had only equivocal, if any, therapeutic effect for pancreatic adenocarcinoma in a randomized double-blind study of 64 patients with locally unresectable carcinoma conducted at the Mayo Clinic, the combination of 5-FU and radiaion was associated with a median survival period of 10.4 months compared to 6.3 months when placebo was combined with roentgen therapy ($p < .05$). Moreover, a recent study of high dose (6000 r) radiation therapy at Duke University suggests increased clinical control and patient survival.

5-Fluorouracil used alone is capable of producing temporary regressions for some patients with advanced pancreatic cancer. In a recent review of the literature in the Cancer Therapy Evaluation Branch, a reported 28% response rate was found among an aggregate of 212 patients. In a single series of 39 patients with biopsy confirmed carcinoma of the pancreas treated at the Mayo Clinic, only a 15% response rate was obtained. These responses were maintained for a median duration of three months. It is apparent that 5-FU has a definite but limited therapeutic potential for advanced pancreatic adenocarcinoma.

The purpose of this study is to compare by controlled evaluation the therapeutic effectiveness of high dose radiotherapy (6000 r) alone and combined radiation therapy, at two dose levels, plus 5-FU in patients with regionally localized but unresectable pancreatic and periampullary carcinoma.

The second group being organized by Cancer Therapy Evaluation Branch is the Testicular Tumor Study Group which consists of urologists, radiotherapists, chemotherapists, and surgeons from Memorial Hospital/Sloan-Kettering Institute, Roswell Park Memorial Institute, M.D. Anderson Hospital and Tumor Institute, Walter Reed Army Hospital,

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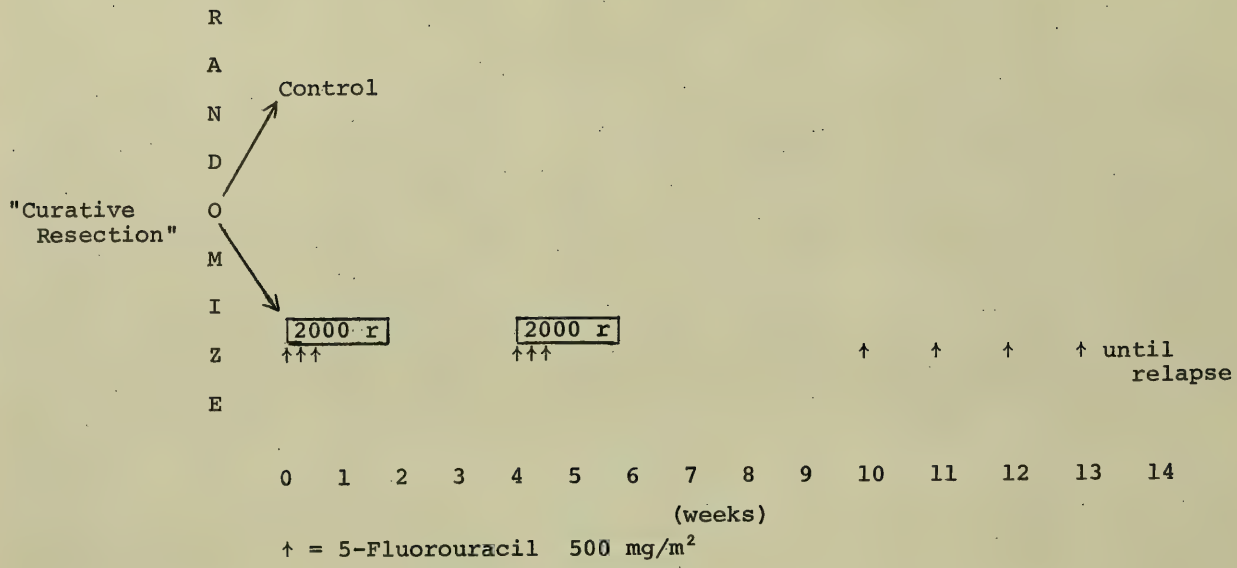

Figure 1. Surgical adjuvant protocol for pancreatic carcinoma. Protocol written by Dr. Charles Haskell (U.C.L.A.) and Dr. Frank Sparks (U.C.L.A.)

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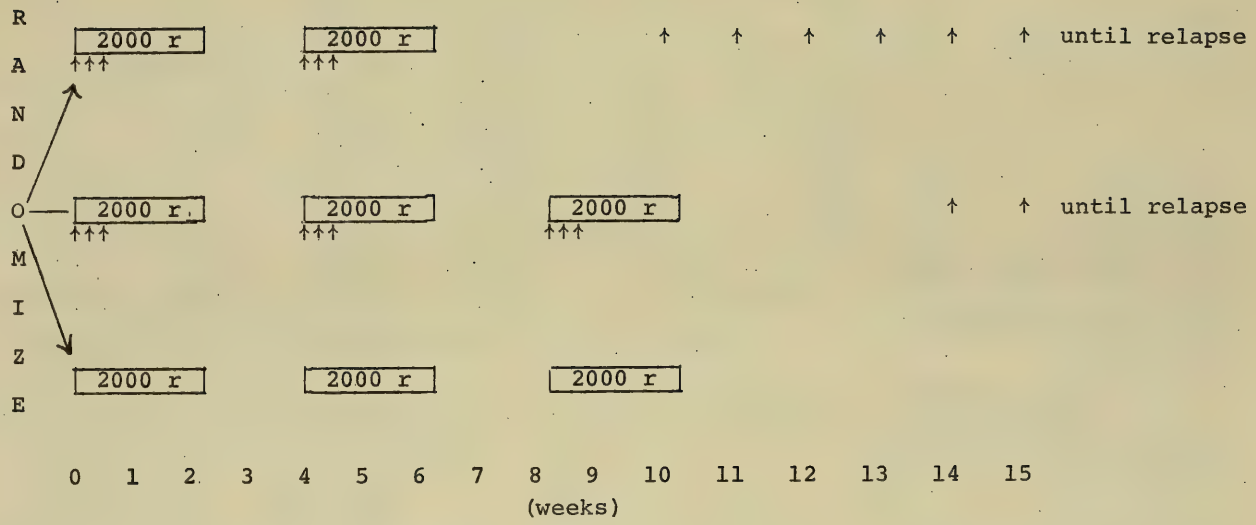
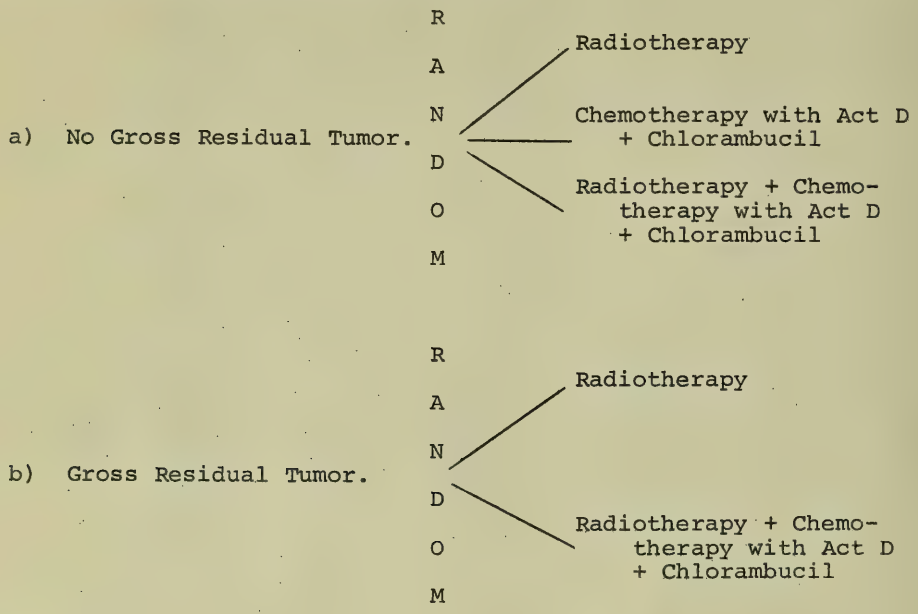


Figure 2. Treatment of locally unresectable pancreatic carcinoma. Protocol written by Dr. Charles Moertel (Mayo Clinic) and Dr. Jacob Lokich (Children's Cancer Research Center, Boston).

Mayo Foundation, and the Bethesda Naval Hospital. This group has developed a protocol for Stage II nonseminomatous tumors of the testicle (Figure 3). The study will compare the length of the disease-free interval in patients with Stage II testicular carcinoma, whose histology is not purely of the seminomatous type. Following "modified" bilateral retroperitoneal lymphadenectomy, two groups of patients will be randomized as follows:



Testicular tumors are relatively rare and account for about one to two percent of all malignant neoplasms in man. Estimates of the incidence of all testicular tumors usually lie between two and three per 100,000 men per year. Unlike most malignant tumors, those of the testicle generally attack men in their prime and the emotional impact of this deadly tumor is great.

Tumors arising from the testis are divided into several histological types, many of which have several subgroups. The nomenclature of the principal neoplasms is chaotic because various authors employ different terminologies and do not always make clear their criteria for defining and naming particular types of tumors. Two of the most important systems used are the Dixon and Moore classification, based on their experience in 900 cases, and the one evolved by Collins and Pugh from 995 cases reviewed by the British Testicular Tumor Panel and Registry. The Testicular Tumor Study Group will use a modification of the Dixon and Moore system, as outlined below:

PROTOCOL FOR ADJUVANT THERAPY OF STAGE II
TESTICULAR CARCINOMA WITH CHEMOTHERAPY (ACTINOMYCIN D AND CHLORAMBUCIL),
RADIATION THERAPY OR CHEMOTHERAPY PLUS RADIATION THERAPY
AFTER RETROPERITONEAL LYMPH NODE DISSECTION

SCHEMA

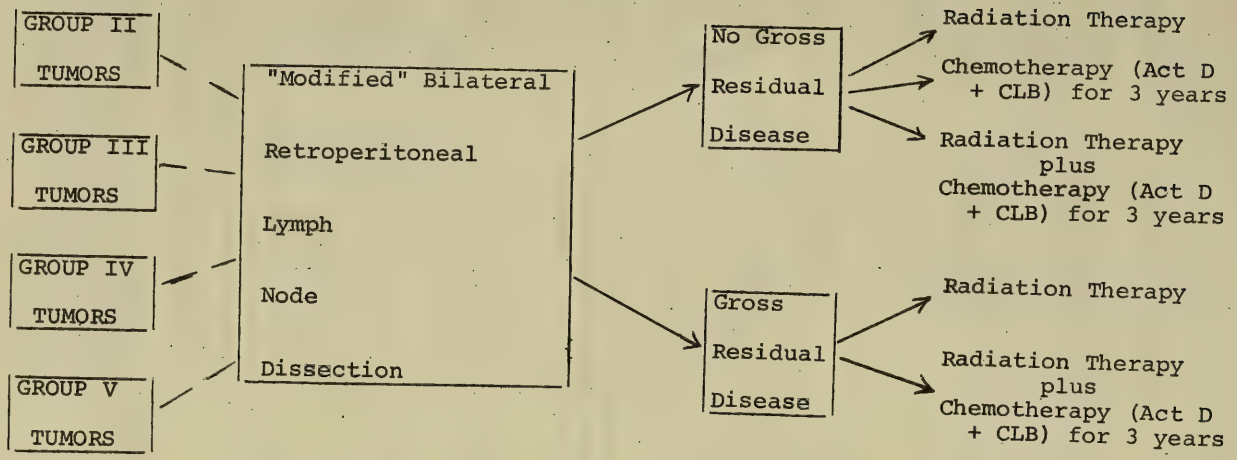

GROUP II TUMORS
GROUP III TUMORS
GROUP IV TUMORS
GROUP V TUMORS
"Modified" Bilateral Retroperitoneal Lymph Node Dissection
No Gross Residual Disease
Gross Residual Disease
Radiation Therapy
Chemotherapy (Act D + CLB) for 3 years
Radiation Therapy plus Chemotherapy (Act D + CLB) for 3 years
Radiation Therapy
Radiation Therapy plus Chemotherapy (Act D + CLB) for 3 years

Figure 3

Tumors of Germ Cell Origin*

Group 1	a)	Classical seminoma
	b)	Spermatocytic seminoma
	c)	Anaplastic seminoma
Group II	a)	Embryonal cell carcinoma, pure or with seminoma
	b)	Yolk sac carcinoma, pure or with embryonal cell carcinoma
Group III		Teratoma, pure or with seminoma
Group IV		Teratoma with embryonal cell carcinoma or choriocarcinoma or both and with or without seminoma
Group V		Choriocarcinoma and embryonal cell carcinoma with or without seminoma

(*Modified from Dixon and Moore - in Atlas of Tumor Pathology, Washington, D.C., Armed Forces Institute of Pathology, 1952 - and from that of Pierce and Abell - in Pathology Annual: 1970. Sheldon Sommers Ed. Appleton Century Crofts, New York).

Clinical staging in testicular tumors is useful as a means of planning treatment and evaluating the efficacy of treatment. For the purposes of this study, the following clinical staging system will be used:

Stage I L±	The tumor is limited to the testis. All retroperitoneal lymph nodes and areolar tissue are free of tumor.
Stage IIa L±	The tumor extends beyond the testis but not beyond the regional draining lymph nodes. No tumor exists in the retroperitoneal areolar tissue.
Stage IIb L±	The tumor extends beyond the testis but not beyond the regional draining lymph node region. Tumor present in the retroperitoneal areolar tissue, with no gross residual tumor remaining after surgery.
Stage IIc L±	The tumor extends beyond the testis but not beyond the regional draining lymph node region. Tumor present in the retroperitoneal areolar tissue with gross residual tumor remaining after surgery.

Stage III Disseminated metastases are present beyond the primary lymphatic drainage or above the diaphram.

L± = Lymphangiogram (positive or negative)

The therapy of testicular tumors has involved the modalities of surgery, radiotherapy and chemotherapy either alone or in various combinations and the optimal therapeutic approach remains to be elucidated. This controversy cannot be resolved until well controlled prospective studies involving sufficiently large numbers of cases with adequate follow-up have been accomplished. Given the relative scarcity of this, tumor controlled prospective studies are almost impossible without a pooling of resources among a group of institutions involved in the therapy of this tumor.

In the large series treated at the Walter Reed Army Medical Center from 1940 to 1968, over 50% of the patients seen had evidence of spread beyond the testis when their full work-up was completed. The initial therapeutic approach to a testicular tumor is a high inguinal orchiectomy which should be performed as soon as there is suspicion of neoplasm in the scrotal contents. This procedure (1) provides an adequate specimen to the pathologist for accurate tissue diagnosis; (2) if performed early, it may prevent metastatic seeding from the primary site to distant sites; and (3) facilitates lymphadenectomy when indicated. Historically, the poor survival from orchiectomy alone has led surgeons to attempt more extensive procedures. Currently, the most commonly accepted surgical procedure is a retroperitoneal lymph node dissection which can be either unilateral or bilateral. The indications for this procedure are all testicular malignancies Stage II or less, except the seminoma group. The rationale for the operation is based on the premise that testicular tumors metastasize by way of the lymphatics to the retroperitoneal nodes which act as a screen for a certain period of time before tumor cells pass on to the thoracic duct and then into the circulation. In the series at Walter Reed, bilateral node dissection has turned up positive nodes in 50 of 76 procedures (67%). With unilateral lymphadnectomy in 137 cases, positive nodes were obtained in 52 cases (38%) and negative nodes in 85 (62%). The yield of positive nodes was higher in the bilateral dissection but, as shown below, when 5 year survival is looked at no difference is seen.

Testicular Tumors - Walter Reed
1940 - 1962
Category II, III, IV, TOTAL

Type of lymphadenectomy and finding	Absolute 5 year Survival TOTAL (Stages I & II)	
Unilateral lymphadenectomy with metastases	23/52	44%

Bilateral lymphadenectomy with metastases	22/52	43%
Unilateral lymphadenectomy without metastases	62/85	76%
Bilateral lymphadenectomy without metastases	19/25	76%
TOTAL	126/213	59%

It is now generally accepted that an additional modality of therapy is required beyond the surgical approach for nonseminomatous disease. In the case of seminoma, the value of radiotherapy is well established and this modality alone is curative for almost all Stage I and II cases. Institutions treating groups II through V histological types (the so-called testicular carcinoma group) all use various combinations and sequences of radiotherapy and chemotherapy in order to improve the survival of this group of patients. The specific aim of the study is to compare in a randomly allocated fashion the increase of the disease-free period achieved in patients with Stage II testicular carcinoma who are treated with either radiotherapy alone, chemotherapy alone, or radiotherapy plus chemotherapy after a standard unilateral retroperitoneal lymphadnectomy.

CTEB staff participates in most of the other disease-oriented approaches ongoing in the DCT and other NCI programs. This includes the participation of the Chief, CTEB, in the Breast Cancer Task Force's Treatment Committee and his assistance given the Associate Director for Medical Oncology in planning and managing the extensive DCT contract program in the breast cancer area. The Chief, CTEB, is also a member of the Lung Cancer Working Party, the Brain Tumor Study Group, the Prostatic Cancer National Program, and the Large Bowel National Program.

The Cancer Therapy Evaluation Branch is responsible for the clinical aspect of international liaison within the Division of Cancer Treatment. The Chief is co-project officer for the contract supported liaison offices in Brussels and Tokyo. In addition, the Chief was a member of the 5-man team, headed by Dr. C. Gordon Zubrod, representing chemotherapy in Moscow in June, 1972 and signed the Joint U.S.-U.S.S.R. Agreement on Chemotherapy, which was the first successful follow-up in the cancer field after President Nixon's historic trip to the Soviet Union. Another visit in December, 1972 cemented the 5-part agreement that has proven both active and successful. Other foreign activities include representation by the Chief on the Scientific Committee of "Biomedicine-Biomedecine", the European Journal of Clinical and Biological Research, and membership on the editorial advisory board of the European Journal of Cancer.

APPENDIX A -- ANALYSIS OF DRUGS TESTED AND RESPONSE IN THE MAJOR SOLID TUMORS AS AN APPROACH TO COMBINED MODALITY THERAPY

Evaluation of the potential of chemotherapeutic agents for use in a successful combined modality thrust in the initial treatment of any tumor requires consideration of two factors: (1) the number of drugs of differing mechanisms of action that are active in the advanced stage of the tumor, and (2) the degree of activity shown by the active agents used alone or in combination. It is the purpose of this review to evaluate these factors for the major solid tumors to attempt a determination of which tumors should have the best chance of responding to a combined modality approach based on the current state of our knowledge. In addition, it is hoped that the analysis will indicate where more studies may be required to develop the tools for making this approach feasible.

The data in this review are from the files of the Cancer Therapy Evaluation Branch and include both published[1] and unpublished material on the 16 solid tumor types and 29 antitumor agents shown in Table 1. All of the 29 drugs chosen have shown definite evidence of activity against at least one tumor type from among the hematological malignancies and solid tumors. Excluded from the review are hormonal agents and investigational agents which have not been adequately evaluated or have shown no evidence of antitumor activity against any tumor.

Table 1 is a broad outline cross-reference chart of the activity of the 29 drugs against the 15 solid tumors. The designation "-" refers to drugs where no data exists. Drug-tumor interactions marked "+" indicate data adequate for making some decision about possible drug activity, although it does not imply complete and adequate evaluation of the drug in the tumor. The notation "±" refers to cases where the data is "barely adequate" for a decision on activity. Evidences of activity that have not been fully established are indicated by "++" and drugs that have clearly established activity are noted "+++". These categories of activity are obviously arbitrary ones but they were established by the CTEB staff through analysis of what is felt to be the maximum data available to any single group experienced in evaluating anticancer drug data.

Table 2 presents a disease-oriented view of the 29 drugs and 16 solid tumors, ranking each tumor on the basis of the percentage of the 29 drugs for which usable activity data is available. It shows that adequate or even barely adequate data (Table 2, A and B) for a decision on the activity of over one-half the 29 drugs is avail-

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[1]Livingston, R.B. and Carter, S.K. Single agents in cancer chemotherapy, Plenum Press, New York, N.Y., 1970.

able for only 4 tumors. These include the 3 most common ones (colon, lung, and breast) and 1 uncommon tumor (melanoma) that is extensively studied because its lesions are readily accessible to measurement and analysis. Among the other 12 tumors, the percentage of drugs with less than adequate data or none at all ranges from 66% to 93% (Table 2, A).

When drugs with a hint of activity (Table 2, B) are added, the same 4 tumors head the list. Except for a 4-point drop in rank for pancreas and a 4-point rise for prostate, there is no significant change in the ranking of the other tumor types. In the 12 remaining tumors, the percentage of drugs totally without data ranges from 55% to 83%.

Table 3A ranks the solid tumors by the percentage of drugs having definitely established activity (+++) in terms of the total number of drugs with at least adequate ($\geq$ +) data. Bladder, sarcoma, and stomach lead the list but with relatively small numbers. In sarcoma, the fact that every drug for which data is available is designated "++" or "+++" reveals a responsiveness to chemotherapy that should indicate the need for evaluating additional drugs. The gynecologic malignancies (ovary and uterine cervix) stand out as potentially responsive and the fact that about 60% of the drugs (Table 2, B) have not been tested in these tumors to a degree allowing a decision on activity should be cause for concern.

Ranking of the tumors by the addition of drugs with evidence (++) of activity (Table 3B) further emphasizes the high degree of activity shown in ovary and cervix. Testicular tumors also are high on the list, reflecting the known sensitivity of this group to chemotherapeutic agents.

Table 4 offers a drug-oriented approach to the data and ranks each drug with respect to the percentage of tumors in which it has been at least adequately evaluated (Table 4, A). Only 8 of the 29 drugs have been adequately evaluated in more than one-half of the 16 tumors. Of the other 21 drugs, the percentage of tumors with less than adequate data or none at all ranges from 56% to 100%. If the tumors with "barely" adequate drug evaluation are included (Table 4, B), 12 of the 29 drugs have data of some use in one-half of the tumors. However, this still leaves 17 drugs where the percentage of tumors totally without data ranges from 56%-100%.

Table 5A ranks the top 15 drugs with definitely established antitumor activity. 5-Fluorouracil is particularly impressive in its clear activity in 7 of 14 tumors tested. The activity of cytoxan and adriamycin also is noteworthy in a significant number (10 +) of tumors.

If the drugs are similarly ranked, but using evidence of activity as well as definite response (Table 5B), the major change is an

upward movement of the nitrosoureas and adriamycin. This is quite understandable in view of the investigational nature of these drugs and the fact that they are still undergoing Phase I and II clinical trials in some of the tumors.

Table 1. Cross reference chart of drug-tumor interactions.

Drugs	Tumor Types															Tumor Activity Spectrum of Drugs				
	Colon	Lung	Breast	Pancreas	Ovary	Prostate	Stomach	Cervix	Head & Neck	Bladder	Kidney	Esophagus	Brain	Testicle	Melanoma	Sarcoma	-	±	+	++	+++
ALKYLATING AGENTS																					
Cytoxan	++	+++	+++	±	+++	±	-	+++	+	±	+	±	+	++	+	+++	1	4	4	2	5
Nitrogen Mustard	+	+++	++	-	±	-	-	±	-	-	-	±	+	++	+	-	7	3	3	2	1
Chlorambucil	+	+	++	-	++	-	-	++	-	±	±	-	-	++	+	++	6	2	3	5	0
Melphalan	+	+	+++	-	+++	-	-	+	-	-	-	-	-	+++	+	-	9	0	4	0	3
Busulfan	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	14	0	2	0	0
ANTI-METABOLITES																					
5-Fluorouracil	+++	+	+++	+++	+++	++	+++	+++	++	+++	+	+	+	-	+	-	2	0	5	2	7
Methotrexate	++	+++	+++	-	-	-	-	+++	+++	-	-	+	++	-	+	-	8	0	2	2	4
6-Mercaptopurine	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	12	0	4	0	0
6-Thioguanine	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	0	1	0	0
Ara-C	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	12	0	4	0	0
MITOTIC INHIBITORS																					
Vincristine	+	+	+++	-	+	±	-	++	-	-	-	-	+	±	+	+++	6	2	5	1	2
Vinblastine	+	+	+++	-	+	-	-	-	-	-	-	-	+	+++	+	-	9	0	5	0	2
ANTITUMOR ANTIBIOTICS																					
Actinomycin D	+	+	+	-	-	-	-	-	-	-	-	-	-	+++	++	+++	10	0	3	1	2
Mithramycin	+	±	+	-	-	±	-	-	-	-	-	-	+	+++	±	-	9	3	3	0	1
Daunomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	0	0	0	0
Adriamycin	+	+++	+++	-	++	++	-	++	++	+++	+	-	-	++	+	+++	4	0	3	5	4
Bleomycin	+	+	+	-	±	-	-	++	+++	-	±	+	±	+++	+	-	5	3	5	1	2
Mitomycin C	+++	++	++	+	-	-	+++	±	-	-	-	±	-	-	±	-	8	3	1	2	2
RANDOM SYNTHETICS & MISCELLANEOUS																					
BCNU	+++	++	++	+	±	-	++	-	±	-	±	-	+++	-	+++	-	6	3	1	3	3
CCNU	+++	++	++	-	-	-	±	-	-	-	±	-	+++	-	++	-	9	2	0	3	2
MeCCNU	+++	++	+	-	-	-	±	-	-	-	±	-	++	-	++	-	9	2	1	3	1
Streptozotocin	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	12	0	4	0	0
DTIC	+	+	+	-	-	-	-	-	-	-	-	-	±	-	+++	++	10	1	3	1	1
Hexamethylmelamine	+	+++	++	-	++	±	±	+	+	±	+	-	-	-	+	-	5	3	5	2	1
Dibromodulcitol	+	+	++	-	-	-	-	-	±	-	-	-	-	-	±	-	11	2	2	1	0
Hydroxyurea	+	++	+	-	-	-	-	±	±	±	+	-	-	-	++	-	8	3	3	2	0
Procarbazine	+	++	+	-	-	-	-	-	-	-	-	-	±	+	+	-	10	1	4	1	0
L-asparaginase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	0	2	0	0
Dibromomannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	0	0	0	0
DRUG ACTIVITY SPECTRUM OF TUMORS																					
-	3	3	5	24	18	22	23	17	20	23	19	23	16	18	6	23					
±	0	1	0	1	3	4	3	3	3	4	5	3	3	1	3	0					
+	19	14	10	3	2	1	0	2	2	0	5	3	6	1	14	0					
++	2	6	7	0	3	2	1	4	2	0	0	0	2	4	4	2					
+++	5	5	7	1	3	0	2	3	2	2	0	0	3	5	2	4					

- = No data

± = Data barely adequate for some decision on possible activity but without the implication that complete and adequate evaluation of the drug in the tumor has taken place

\+ = Data adequate to the above decision process

++ = Evidence of clinical activity, although not clearly established

+++ = Definitely established clinical activity

Table 2. Ranking of tumor types on the basis of the percentage of drugs with at least (A) adequate data or (B) barely adequate data.

A. Adequate data*

Rank	Tumor Type	No. drugs $\geq$ + / Total (29)	%
1	Colon	26/29	90%
2	Lung	25/29	86%
3	Breast	24/29	83%
4	Melanoma	20/29	69%
5	Brain	10/29	34%
6	Testicle	10/29	34%
7	Cervix	9/29	31%
8	Ovary	8/29	28%
9	Head & Neck	6/29	21%
10	Sarcoma	6/29	21%
11	Kidney	5/29	17%
12	Pancreas	4/29	14%
13	Stomach	3/29	10%
14	Esophagus	3/29	10%
15	Prostate	3/29	10%
16	Bladder	2/29	7%

B. Barely adequate data**

Rank	Tumor Type	No. drugs $\geq$ ± / Total (29)	%
1	Colon	26/29	90%
2	Lung	26/29	90%
3	Breast	24/29	83%
4	Melanoma	23/29	79%
5	Brain	13/29	45%
6	Cervix	12/29	41%
7	Testicle	11/29	38%
8	Ovary	11/29	38%
9	Kidney	10/29	34%
10	Head & Neck	9/29	31%
11	Prostate	7/29	24%
12	Sarcoma	6/29	21%
13	Stomach	6/29	21%
14	Esophagus	6/29	21%
15	Bladder	6/29	21%
16	Pancreas	5/29	17%

* Includes drugs rated +, ++, and +++ in Table 1.

** Includes drugs rated ±, +, ++, and +++ in Table 1.

Table 3. Ranking of solid tumors by the percentage of drugs showing (A) definitely established activity or at least (B) evidence of activity.

A. Definite activity*

Rank	Tumor Type	No. drugs +++ / No. drugs ≥ +	%
1	Bladder	2/2	100%
2	Sarcoma	4/6	66%
3	Stomach	2/3	66%
4	Testicle	5/10	50%
5	Ovary	3/8	37%
6	Cervix	3/9	33%
7	Head & Neck	2/6	33%
8	Breast	7/24	29%
9	Pancreas	1/4	25%
10	Lung	5/25	20%
11	Brain	2/10	20%
12	Colon	5/26	19%
13	Melanoma	2/20	10%
14	Esophagus	0/3	0
15	Kidney	0/5	0
16	Prostate	0/3	0

B. Evidence of activity**

Rank	Tumor Type	No. drugs ≥ ++ / No. drugs ≥ +	%
1	Bladder	2/2	100%
2	Sarcoma	6/6	100%
3	Stomach	3/3	100%
4	Testicle	9/10	90%
5	Cervix	7/9	78%
6	Ovary	6/8	75%
7	Head & Neck	4/6	66%
8	Prostate	2/3	66%
9	Breast	14/24	58%
10	Lung	11/25	44%
11	Brain	4/10	40%
12	Melanoma	6/20	30%
13	Colon	7/26	27%
14	Pancreas	1/4	25%
15	Kidney	0/5	0
16	Esophagus	0/3	0

* Includes drugs rated +++ in Table 1 in terms of the total rated + and above.

** Includes drugs rated ++ and +++ in Table 1 in terms of the total rated + and above.

Table 4. Ranking of drugs on the basis of the percentage of tumors with at least (A) adequate or (B) barely adequate data.

A. Adequate data*

Rank	Drug	No. tumors $\geq$ + / Total (16)	%
1	5-Fluorouracil	14/16	87%
2	Adriamycin	12/16	75%
3	Cytoxan	11/16	69%
4	Chlorambucil	8/16	50%
5	Methotrexate	8/16	50%
6	Vincristine	8/16	50%
7	Bleomycin	8/16	50%
8	Hexamethylmelamine	8/16	50%
9	Melphalan	7/16	44%
10	Vinblastine	7/16	44%
11	BCNU	7/16	44%
12	Nitrogen mustard	6/16	37%
13	Actinomycin D	6/16	37%
14	Mitomycin C	5/16	31%
15	CCNU	5/16	31%
16	MeCCNU	5/16	31%
17	DTIC	5/16	31%
18	Hydroxyurea	5/16	31%
19	Procarbazine	5/16	31%
20	6-Mercaptopurine	4/16	25%
21	Ara-C	4/16	25%
22	Mithramycin	4/16	25%
23	Streptozotocin	4/16	25%
24	Dibromodulcitol	3/16	19%
25	Bulsulfan	2/16	12%
26	L-Asparaginase	2/16	12%
27	6-Thioguanine	1/16	6%
28	Daunomycin	0/16	0
29	Dibromomannitol	0/16	0

B. Barely adequate data**

Rank	Drug	No. tumors $\geq$ ± / Total (16)	%
1	Cytoxan	15/16	94%
2	5-Fluorouracil	14/16	87%
3	Adriamycin	12/16	75%
4	Bleomycin	11/16	69%
5	Hexamethylmelamine	11/16	69%
6	Chlorambucil	10/16	62%
7	Vincristine	10/16	62%
8	BCNU	10/16	62%
9	Nitrogen mustard	9/16	56%
10	Methotrexate	8/16	50%
11	Mitomycin C	8/16	50%
12	Hydroxyurea	8/16	50%
13	Melphalan	7/16	44%
14	Vinblastine	7/16	44%
15	CCNU	7/16	44%
16	MeCCNU	7/16	44%
17	Mithramycin	7/16	44%
18	Actinomycin D	6/16	37%
19	DTIC	6/16	37%
20	Procarbazine	6/16	37%
21	Dibromodulcitol	5/16	31%
22	6-Mercaptopurine	4/16	25%
23	Ara-C	4/16	25%
24	Streptozotocin	4/16	25%
25	Busulfan	2/16	12%
26	L-Asparaginase	2/16	12%
27	6-Thioguanine	1/16	6%
28	Daunomycin	0/16	0
29	Dibromomannitol	0/16	0

* Includes drugs rated +, ++, and +++ in Table 1.

** Includes drugs rated ±, +, ++, and +++ in Table 1.

Table 5. Ranking of the top 15 drugs based on (A) definitely established activity or at least (B) evidence of activity.

A. Definite activity*

Rank	Drug	No. tumors +++ / No. tumors ≧ +	%
1	5-Fluorouracil	7/14	50%
2	Methotrexate	4/8	50%
3	Cytoxan	5/11	45%
4	Melphalan	3/7	43%
5	BCNU	3/7	43%
6	Mitomycin C	2/5	40%
7	CCNU	2/5	40%
8	Adriamycin	4/12	33%
9	Actinomycin D	2/6	33%
10	Vinblastine	2/7	28%
11	Vincristine	2/8	25%
12	Bleomycin	2/8	25%
13	Mithramycin	1/4	25%
14	MeCCNU	1/5	20%
15	DTIC	1/5	20%

B. Evidence of activity*

Rank	Drug	No. tumors ≧ ++ / No. tumors ≧ +	%
1	CCNU	5/5	100%
2	BCNU	6/7	86%
3	MeCCNU	4/5	80%
4	Mitomycin C	4/5	80%
5	Adriamycin	9/12	75%
6	Methotrexate	6/8	75%
7	5-Fluorouracil	9/14	64%
8	Cytoxan	7/11	64%
9	Actinomycin D	3/6	50%
10	Nitrogen mustard	3/6	50%
11	Melphalan	3/7	43%
12	DTIC	2/5	40%
13	Vincristine	3/8	37%
14	Bleomycin	3/8	37%
15	Hexamethylmelamine	3/8	37%

* Includes tumors rated +++ per Table 1 in terms of the total rated + and above.

** Includes tumors rated ++ and +++ in Table 1 in terms of the total rated + and above.

APPENDIX B -- REVIEW OF DRUGS IN PHASE I AND II CLINICAL TRIALS

DRUGS IN PHASE I CLINICAL TRIALS

New investigational drugs which have been studied only in Phase I clinical trials are:

1. Chromomycin A_3 (NSC 53514)
2. d-Tetrandrine (NSC 77037)
3. Thalicarpine (NSC 68075)
4. VP 16-213 (NSC 141540)
5. ICRF 159 (NSC 129943)
6. Yoshi 864 (NSC 102627)

I. CHROMOMYCIN A_3

A. Mode of action.

1. Inhibition of DNA dependent RNA synthesis (RNA Polymerase). Presence of a) guanine base b) Mg++ is required for the inhibition.
2. To lesser extent inhibition of DNA synthesis (DNA Polymerase).

B. Animal antitumor effect.

1. Screening:
   a. Active against Leukemia P 388 in mice, sarcoma 180, lymphatic leukemia SN 36, Yoshida sarcoma, hepatoma AH 130.
   b. Inactive against leukemia L1210 in mice.
2. Schedule dependency: (P 388) daily schedule most active.

C. Pharmacokinetic studies.

Species: Rabbit

1. Plasma half-life - about 30'
2. Urinary excretion - 36% within 3 hours
3. Biliary excretion - 1.9% of administered dose in 3 hours
4. Plasma protein binding - negative
5. High organ tissue concentration - lymph glands, liver, and kidney
6. Crossing blood brain barrier - possible, not proven

E. Toxicity.

1. Single dose (IV)
   a. mice LD 50 1.85 mg/kg (5.5 mg/m^2)
   b. rabbit MTD 0.1 mg/kg (1.1 mg/m^2)

c. dog MTD 0.1 mg/kg (2.0 mg/m^2)
d. monkey MTD 0.165mg/kg (1.98 mg/m^2)

2. Repeated dose - daily x 5 IV.
a. mouse LD 50 0.6 mg/kg (1.8 mg/m^2)
b. dog TDL 0.025 mg/kg (0.5 mg/m^2)
c. monkey TDL 0.025 mg/kg (0.3 mg/m^2)

Hematological, GI, liver, renal hemorrhagic syndrome, hypocalcemia.

E. Phase I studies.

Chromomycin A_3 has been investigated in 4 separate Phase I studies, using 3 different dose schedules: single IV, daily x 5 IV, and daily x 10 IV. The maximum tolerated dose has been established only for the daily x 5 IV schedule (0.9 mg/m^2). The dose limiting toxicity is renal and occurs whenever the dose is escalated above this level. Hypocalcemia was another finding that has not been previously observed in the Japanese and South African studies. The MTD for the other schedules has not been reached.

Preliminary results of pharmocokinetic studies in man, included in Morgan's Phase I study, appear to correspond with the findings in animals. There is a particularly short plasma half-life and rapid urinary excretion within 48 hours.

The Phase I studies on Chromomycin A_3 are summarized in Figure 1.

II. D-TETRANDRINE.

A. Mode of action - unknown.

B. Animal antitumor effect.

1. Screening: Active in Walter 256; inactive in L1210 in mice.
2. Schedule dependency: Single and daily doses IP equally active.

C. Pharmacokinetic studies - no data available.

D. Toxicity.

1. Single dose (IV) - no data available.
2. Repeated dose - daily x 5 IV infusion.

	DOG		MONKEY	
HNTD	10 mg/kg	200 mg/m^2	10 mg/kg	120 mg/m^2
TDL	25 mg/kg	500 mg/m^2	25 mg/kg	300 mg/m^2
TDH	50 mg/kg	1000 mg/m^2	50 mg/kg	600 mg/m^2
LD	100 mg/kg	2000 mg/m^2	100 mg/kg	1200 mg/m^2

Cardiovascular, Respiratory, Hematopoetic local reaction at site of injection, Kidney, Liver.

E. Phase I studies.

D-tetrandrine recently entered Phase I study and it is too early for analysis of the data or for any conclusions. The study now in progress is summarized in Figure 2.

III. THALICARPINE.

A. Mode of action - not fully elucidated.

Inhibition of:
1. DNA synthesis (DNA polymerase)
2. RNA synthesis (incorporation of uridine in RNA)
3. Protein synthesis

B. Animal antitumor effect.

1. Screening: active in Walker 256; inactive in L1210
2. Schedule dependency: Walker 256 - intermittent schedules appear to be superior to daily schedules.

C. Pharmacokinetic studies.

Species: Dog

1. Plasma half-life: rapid clearence from plasma
2. Urinary excretion: no data
3. Biliary excretion - no data
4. Plasma protein binding - no data
5. High organ tissue concentration - liver, kidney, lung, pancreas, and spleen
6. Crossing blood brain barrier - possible; concentration of the drug in the brain of females higher than that of males.

D. Toxicity.

1. Single dose (IV)

	LD50			DOG		MONKEY	
Species	mg/kg	mg/m^2		mg/kg	mg/m^2	mg/kg	mg/m^2
Mouse (IP)	247	731	HNTD	-	-	-	-
Rat	500	3000	TDL	15.6	212	-	-
			TDH	31.3	626	-	-
			LD	62.6	1252	31.3	375.6

2. Repeated dose.

	Dog (daily x 5)		Monkey (Daily x 14)	
	mg/kg	mg/m^2	mg/kg	mg/m^2
HNTD	5.2	114	-	-

TDL	10.4	208	-	-
TDH	20.8	416	5.2	62.4
LD	41.6	832	10.4	120.8

Neurotoxic (convusions and Ataxia), hepatotoxic, nephrotoxic, pulmonary toxicity, cardiotoxicity.

E. Phase I studies.

Thalicarpine recently entered Phase I study and it is too early for analysis of the data for any conclusions. The study now in progress is summarized in Figure 3.

IV. VP 16-213

A. Mode of action.

1. Mitotic arrest at metaphase.
2. Prevention of cells from entering mitosis.
3. Inhibition of H_3 thymidine and uridine uptake; may also affect H_3 leucine uptake.

B. Animal antitumor effect.

1. Active in sarcoma 180 and 37, Walker carcinosarcoma and Erlichs ascites tumor.
2. Curative in L1210, 42/45 cures at 30 mg/kg IP twice weekly.
3. Little or no activity in intracerebral L1210 (ILS% ≃25)

C. Pharmacokinetic studies.

1. Protein bound.
2. Evenly distributed through body (rats) little cerebral uptake.
3. Excreted (in rats) via bile and urine, the former predominating.

D. Animal toxicology.

Rats and monkeys - myelosuppresion, lymphoid involution (no MTD given).

E. Clinical dosage schedules.

1. 45 $mg/m^2/d$ x 7
2. 86 mg/m^2 biw x 3 weeks cut to 54 mg/m^2 for more prolonged duration of therapy.
3. 60 $mg/m^2/d$ q 21 d (2-6 cycles)

All via 30-60 minute IV infusion.

F. Dosage formulation.

1. Solvent system containing Tween 80, polyethylene glycol, benzyl alcohol, citric acid, and ethanol.
2. Oral preparation under investigation in Europe.

G. Clinical toxicity.

1. Leukopenia, thrombocytopenia (reversible) with nadir 12-16 days after onset of therapy on the daily x 7 schedule.
2. Alopecia
3. Nausea and vomiting
4. Hypotension - uncommon

H. Antitumor effect: activity seen in lymphomas, acute monocytic and myelomonocytic leukemia.

I. Phase I studies.

Two phase I studies are in progress for VP-16, using either 30 minute IV infusion once a week for 6 weeks (Selawry) or a twice weekly schedule. The weekly dose has been escalated to 100 mg/m^2 but the maximum tolerated dose has not been established. Hematologic and GI toxicity appear to be major side effects, while alopecia, fever, hypotension, and allergy are also reported. These studies are summarized in Figure 4.

V. ICRF 159.

A. Mode of action.

1. Inhibition DNA synthesis
2. Chelation of divalent cations
3. Alkylation
4. Inhibition G_2-M phase of cell growth
5. Prevention of metastases formation through effect on blood vessels.

B. Animal antitumor effect.

1. Screening: Active in L1210 and Lewis lung
2. Schedule dependency: Intermittent schedule most active

C. Pharmacokinetic studies.

Species: Sarcoma 180 and mice

1. Plasma half-life - no data
2. Urinary excretion - +
3. Biliary excretion - +

4. Plasma protein binding - no data
5. High organ tissue concentration - liver and kidney
6. Crossing blood brain barrier - no data

D. Toxicity.

1. Single dose (PO)

		mg/kg	mg/m^2
Dog	HNTD	320	6400
	TDL	1000	20,000
	TDH	-	-
	LD	-	-

2. Repeated dose (Daily x 5, PO)

	Dog		Monkey	
	mg/kg	mg/m^2	mg/kg	mg/m^2
HNTD	-	-	-	-
TDL	20	400	20	240
TDH	160	3200	-	-
LD	320	6400	-	-

3. Qualitative.

a. GI - anorexia, vomiting, diarrhea
b. Hematological - leukopenia, thrombocytopenia
c. Hepatic - SGOT, SGPT ↑
d. Renal - BUN ↑
e. Testicular - degeneration and hypospermia

E. Phase I studies.

Two studies with ICRF 159 have been completed. Based on the pharmacokinetic study, the recommended dose is 3000 mg/m^2 (PO) given once weekly. Leukopenia and GI toxicity are the major side effects. These studies are summarized on Figure 5.

VI. Yoshi 864.

A. Mode of action.

1. Undetermined
2. Originally synthesized in Japan as an agent for tumors resistant to alkylators.
3. Increase of glycogen and oligosaccharide content in the tumor tissue.

B. Animal antitumor effect.

1. Screening: Active in L1210 leukemia in mice and against Nitrogen mustard-resistant AH-7974, AH-66, and SN-36.

2. Schedule dependency: intermittent schedule IP days 1, 5, and 9 most active.

C. Pharmacokinetic studies - no data available.

D. Toxicity.

1. Single dose (IV)

Mouse LD50 111 mg/kg (333 mg/m^2) - male
140 mg/kg (420 mg/m^2) - female

2. Repeated dose (Daily x 5 IV)

		(male)	(female)
Mouse	LD50	70 mg/kg (210 mg/m^2)	81 mg/kg (243 mg/m^2)
Dog	MTD	2.5 mg/kg (50 mg/m^2)	

3. Qualitative.

a. Hematologic
b. Hepatic
c. Renal

E. Phase I studies.

Yoshi 864 has been studied only by the Central Oncology Group. Using the daily x 5 IV push schedule at present, the dose has been escalated to 2.2 mg/kg (81.4 mg/m^2). Leukocytopenia and thrombocytopenia are reported when daily dose is above 1.5 mg/kg (55.5 mg/m^2). The COG study is summarized on Figure 6.

Study	# Pt. Eval.	Dose Schedule	Dose mg/m^2			Incidence of Toxicity (%)				
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.	Hepato.	Renal*	Hypocal-cemia
Kovach et al, Mayo Clinic	26	Daily x 5 IV	0.5	1.1	0.9	55.2	13.1	18.4	26.3*	18.4
Vaitkevi-cius et al SWG	23	Daily x 5 IV, 30-60' 5% glucose infusion	0.25	1.5	0.75><1.0	47.8	0	0	26.0*	13.1
Hoeltgen St. Lukes, Chicago	12	Daily x 10 IV	0.1	0.4						
Morgan** Tulane, New Orleans	3	Single IV	0.5							

* Dose limiting toxicity
** Pharmacokinetic study included

Figure 1. Phase I Studies with Chromomycin A_3 (NSC 58514).

Study	# Pts. Eval.	Dose Schedule	Dose mg/m^2			Incidence of Toxicity (%)				
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.	Hepato.	Renal	Hypocal-cemia
De Conti ECOG 1773		Single IV infusion	50							
		Daily x 5 IV infu-sion	50							

Figure 2. Phase I Studies with d-Tetrandrine (NSC 77037).

Study	# Pts. Eval.	Dose Schedule	Dose mg/m^2			Incidence of Toxicity (%)		
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.	Hepato.
Creaven 10510	6	Single IV 1 hr. infusion 500 ml D5/W	100	400				

Figure 3. Phase I Studies with Thalicarpine (NSC 68075).

Study	Pts. # Eval	Dose Schedule	Dose mg/m^2			Incidence of Toxicity (%)				
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.	Alo-pecia	Allergy Fever	Hypo-tension
Selawry 10630	14	30' IV infusion in 250-500 ml saline once a week	20	100			28.5			7.1
Young (SKI)	71*	Twice a week		100*						
				300**		17	85	30	9	
		Once a week		100***						

* Dose for solid tumors
** Dose for leukemia
*** Dose for reduced bone marrow reserve

Figure 4. Phase I Studies with VP-16 (NSC 141540).

Study	# Pts. Eval.	Schedule	Dose mg/m^2			Incidence of Toxicity (%)				
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.* WBC< 5000	Hepto.	Derma-titis	Alo-pecia
Creaven ** 5813	26	Single PO	1000	10500		7	61.5			
	6	Single PO weekly x 6		3000***						
	4	Split TID once wkly		3000						
Bellet et al 8727	10	Daily x 3 PO each daily dose provided in 3 equal portions given in 8 hr. intervals	250	1250	1000	50	70		20	10

* Dose limiting toxicity
** Pharmacokinetic study included
*** Dose recommended for Phase II studies

Figure 5. Phase I Studies with ICRF 159 (NSC 129943).

Study	# Pts. Eval.	Dose Schedule	Dose mg/m^2			Incidence of Toxicity (%)			
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.	Hepato.	Renal
Altman, COG 7111	20	Daily x 5 IV push	0.25 mg/kg (9.25)	2.2 mg/kg (81.4)		+	+ Leuko-penia + Throm-bocyto-penia when dose >1.5 mg/kg	-	-

Figure 6. Phase I Studies with Yoshi 864 (NSC 102627).

DRUGS IN PHASE I AND II CLINICAL TRIALS

I. 5-AZACYTIDINE (NSC 102816)

Six different dose schedules have been studied in Phase I trials: single weekly dose x 4, twice weekly x 8, daily x 5 repeated after 9, 14, or 21 days, and, finally, daily x 10. The maximum tolerated doses appear to be 500 mg/m^2 for the once weekly schedule, 150 mg/m^2 for the twice weekly schedule, 150-200 mg/m^2 for the daily x 5 schedules, and 60 mg/m^2 for the daily x 10 schedule.

The hematological toxicities are dose-limiting and include delayed leukocytopenia and thrombocytopenia. GI toxicity consists of severe nausea, vomiting, anorexia, and diarrhea. Furthermore, fever, pruritic folicular skin rash, and elevation of serum transaminases are reported. Two of the five studies include pharmacokinetic studies. They revealed a plasma half-life of 3.5 hours (range 3-4.7 hours) and excretion of 85-90% of radiolabelled drug or its metabolites in urine within 24-48 hours. A low level of radioactivity was also detected in the cerebrospinal fluid with the maximum 24 hours after the IV administration. Figure 7 summarizes Phase I studies.

In Phase I and in Phase II studies, in which nearly all used daily schedules, the drug showed significant activity against acute myeloblastic leukemia in children and adults (34% response). The results in acute lymphoblastic leukemia and solid tumors do not confirm the original promising reports from Czechoslovakia and the Central Oncology Group. However, more data are needed to reach final conclusions. A summary of Phase II results is given in Figure 8.

II. ISOPHOSPHAMIDE (NSC 109724)

Phase I study (Figure 9) by Dr. Cohen in the NCI-VA Medical Oncology Service reached the maximum tolerated single dose of 5000 mg/m^2 IV. The dose recommended for Phase II trial ranges between 3800 and 5000 mg/m^2. The pharmacokinetic studies revealed peak urinary alkylating activity continuing for 3 days.

Phase II results presently available in 2 signle tumors, adenocarcinoma of the breast and the colon, do not suggest superiority of this analogue over cytoxan (Figure 10). There is an apparent need for controlled randomized Phase II studies comparing the effect of both drugs in all signal tumors. However, isophosphamide is not too appealing since, in addition to the toxicities it has in common with cytoxan, it produces more severe hematuria caused by hemorrhagic cystitis.

III. CIS-PLATINUM (II) DIAMMINODICHLORIDE (NSC 119875).

In Phase I trial various dose schedules were used: single IV dose repeated every 3 weeks, twice weekly IV doses repeated every 3-4 weeks, daily x 5 IV doses repeated every 3 weeks, and daily IV push given until toxicity. The toxicity reported includes predictable and reversible myelosuppression, reversible renal insufficiency, high frequency ototoxicity detectable by audiometry, and GI intolerance.

The maximum tolerated dose of the platinum drug is 20-25 mg/m^2/d x 5 or 75-100 mg/m^2 as a single dose. Pharmacokinetic studies in man show that platinum is rapidly removed from the circulation and widely distributed in the tissues, less than 10% of the platinum remaining in the plasma at 1 hour. The initial half-life ranges between 41-49 minutes, while the secondary one is between 58.5-73 hours. About 90% of the drug in plasma appears to be protein bound and 19.2-33.9% of the administered drug is excreted in the urine within 24 hours and 25-43.6% in 96 hours.

While hints of antitumor activity have been observed in the initial clinical evaluations, fully disease-oriented phase II trials have been slow in developing. Currently, a trial is nearing completion in GI cancer at the Mayo Clinic, using 50 mg/m^2 single IV dose. While the preliminary results are not encouraging, many patients are still on study. Broader Phase II trials in other diseases are now being planned, as are a series of combination studies evolving out of the experimental data in leukemia L1210 indicating that platinum is synergistic with 5-fluorouracil, Cytoxan, and other drugs.

Study	# Pts. Eval.	Dose Schedule	Dose (mg/m^2) Start	Highest Escalation	Maximum Tolerated	Incidence of Toxicity (%) GI	Hem.	Hepato. SGOT ↑ <40 unit	Fever	Pruritic Folicular Skin Rash
McCreadey 51-17	39[b]	Daily x 5 IV q 21 d	2.2	275	225	75	60*	-	Occas-sional	
	12[c]	Daily x 5 IV q 14 d		400	300				Common	
Karon CCA 020	37[d]	Daily x 5 IV q 14 d	2.0	200	150 - 200	Most	+ *	-		50%
Weiss[a] COG 7110	30	Daily x 10 IV	0.03/kg (1.11)	2.4/kg (88)	1.6/kg (59.2)	83.3	90*	23.3		6.6
Vogler[a] SEG 137	24	Twice a wk IV x 8 wks	50	200	150	87.5*	29.5 WBC <1500	-	-	-
Shnider EST 471	15	Weekly IV x 4	200	633	500	100*	33.3	-	-	-
	12	Daily x 5 9 days off repeat	50	163	150	100* Nausea Vomit-ing, Diarr-hea	50* Leuko-penia Throm-bocyto-penia	-	-	-

* Dose limiting toxicity
[a] Pharmacokinetic study included
[b] Solid tumors [c] Acute leukemia
[d] Children - AML & ALL

Figure 7. Phase I studies with 5-Azacydidine (NSC 102816).

Tumor Type	# Pts. Eval.	# CR	# CR + PR	% Response	Investigator
Breast Carcinoma	27	0	8	29.6	Weiss, 1972; Dao, 1972
Colon Carcinoma	32	0	2	6.2	Weiss, 1972; Moertel, 1972
Bronchogenic Carcinoma	4	0	0	0	Weiss, 1972; COG 7123
Pancreatic Carcinoma	2	0	0	0	Weiss, 1972; COG 7123
Ovarian Carcinoma	1	0	1		Weiss, 1972
Malignant Melanoma	22	0	3	13.6	McCreadey 5117
AML	44	8	15	34	Freireich, 1973; Karon, 1973; Vogler, 1973; Tan, 1973
ALL	39	2	4	10.2	Freireich, 1973; Karon, 1973; Tan, 1973
Malignant Lymphoma	2	0	1		Weiss, 1973; McCreadey
Malignant Gliomas	0	0	0		

Figure 8. Phase II Studies with 5-Azacytidine (NSC 102816).

Study	# Pts. Eval.	Dose Schedule	Dose (mg/m^2)			Incidence of Toxicity (%)				
			Start	Highest Escalation	Maximum Tolerated	GI	Hem.	Hepato.	Haema-ture	CNS
Cohen *	39	Single IV	200	5000	5000	41.3	15.3	-	25.6	5.1

* Pharmacokinetic studies included

Figure 9. Phase I studies with Isophosphamide (NSC 109724).

Tumor Type	# Pts. Eval.	# CR	# CR + PR	% Response	Investigator
Breast Carcinoma	20*		4	20	Ahman, 1973
Colon Carcinoma	33**		1	3	Kovach, 1973

* 4000 mg/m^2 IV every 4 weeks
** 4000 mg/m^2 IV every 3 weeks

Figure 10. Phase II Studies with Isophosphamide (NSC 109724).

DRUGS IN PHASE II CLINICAL TRIALS

I. ADRIAMYCIN (NSC 123127)

The Cancer Therapy Evaluation Branch has accumulated data on over 1,800 cancer cases treated with adriamycin alone, and the total experience including combination usage is more than 2,000 cases. This rapid accrual of data indicates the widespread clinical interest in this drug and would be even more extensive were it not for a production problem in Italy which limited U.S. clinical studies for 5 months in late 1972.

Adriamycin has demonstrated definite activity against at least 10 major kinds of cancer. These 10 encompass all of the major types including adult solid tumors (breast, lung, sarcomas), hematologic malignancies (malignant lymphoma, acute leukemia), and childhood solid tumors (Figure 11). In addition, adriamycin shows evidence of antineoplastic activity against other adult solid tumors, but the data requires confirmation by further experience which will increase the number of patients studied.

The most serious toxic side effect of adriamycin is cardiac toxicity which is related to the total dose of drug administered and begins to become a danger when a total dose of 550 mg/m^2 is exceeded.

II. DIBROMODULCITOL (NSC 104800).

A Phase II study of dibromodulcitol administered at 3.5 mg/kg in divided daily doses (PO) for 42 days has been carried out by the Central Oncology Group. The primary toxicities noted were thrombocytopenia and leukopenia which often required the discontinuation of the drug. Objective response of measurable lesions was observed in six of the signal tumor types as summarized in Figure 12.

Phase II studies against malignant gliomas, as well as acute and chronic leukemias, are under way.

III. METHYL CCNU(NSC 95441)

Phase II studies on MeCCNU are underway in a number of institutions and cooperative groups. The data accumulated by the Cancer Therapy Evaluation Branch in more than 300 patients reveals that MeCCNU appears promising against adenocarcinoma of the colon, malignant gliomas and squamous cell carcinomas of different origins (Figure 13). However, the numbers are still small and more data will be needed to confirm these findings.

Tumor Type	# Pts. Eval.	# CR	# CR + PR	% Response
Breast Carcinoma	183	1	67	36.6
GI Carcinoma	92		8	8.7
Bronchogenic Ca.	314		53	16.9
Ovarian Carcinoma	48	1	18	37.5
Malignant Melanoma	27	0	0	0
AML	56	5	20	35.7
ALL	144	36	57	39.6
Malignant Lymphoma	222	9	92	41.4
Soft Tissue and Bone Sarcomas	234	3	57	24.4
Childhood Solid Tumors	180	11	77	42.8
a. Neuroblastoma	89	3	28	31.5
b. Wilms' Tumor	40	6	26	65
c. Ewing's Sarcoma	51	2	23	45.1
Testicular Tumors	58		11	19
Bladder Cancer	93		26	28
Cervix Cancer	28	1	9	31.1
Prostate	14		3	21.4
Kidney	56		4	7.1
Head & Neck	76		10	13.2
Thyroid Cancer	22		10	45.5
TOTALS	1847	67	522	28.3

Figure 11. Phase II Studies with Adriamycin (NSC 123126).

Tumor Type	# Pts. Eval.	# CR	# CR + PR	% Response
Breast Carcinoma	34	1	12	35.3
Colon Carcinoma	28	1	4	14.3
Bronchogenic Ca.	43	0	8	18.6
Ovarian Carcinoma	3	0	1	33.3
Malignant Melanoma	21	1	5	23.3
Malignant Lymphoma	8	0	3	37.5

Figure 12. Phase II Studies with Dibromodulcitol (NSC 104800).

Tumor Type	# Pts. Eval.	# CR	# CR + PR	% Response
Breast Carcinoma	33	0	2	6.1
Colon Carcinoma	77	4	12	15.6
Bronchogenic Ca.	107	1	16	15.0
Pancreatic Carcinoma	10	0	0	0
Ovarian Carcinoma	5	0	0	0
Malignant Melanoma	48	1	9	18.8
Malignant Lymphoma	21	1	3	14.3
Malignant Gliomas	32	0	9	28.1
Head & Neck	25	0	3	12.0
Cervix	5	0	2	40.0
TOTALS	363	7	56	15.4

Figure 13. Phase II Studies with MeCCNU (NSC 95441).

HONORS AND AWARDS

1. Carter, S.K. K.D.A. Allen Visiting Professor of Oncology at Presbyterian Medical Center, Denver, Colorado, August 21-25, 1972.

2. Carter, S.K. Elected to alumni membership in New York Medical College chapter of Alpha Omega Alpha.

3. Carter, S.K. Appointed to Scientific Committee of Biomedicine-Biomedecine, The European Journal of Clinical and Biological Research.

4. Carter, S.K. Member Program Committee ACS-NCI National Conference on Cancer Chemotherapy.

PUBLICATIONS

1. Carter, S.K.: The chemotherapeutic approach to cancer therapy: A quick overview. In Clark, R.L. and Cumley, R.W. (Eds.): Year Book of Cancer 1972. Chicago, Year Book Medical Publishers, 1972, pp. 475-498.

2. Carter, S.K.: Current status of new agents. Cancer Chemother. Rep. 3: 33-47, 1972.

3. Carter, S.K.: New drugs on the horizon in bronchogenic carcinoma. Cancer 30: 1402-1409, 1972.

4. Carter, S.K.: The search for therapeutic cell controls by the chemotherapy program of the National Cancer Institute. J. Invest. Derm. 59: 128-138, 1972.

5. Carter, S.K.: Single and combination nonhormonal chemotherapy in breast cancer. Cancer 30: 1543-1555, 1972.

6. Carter, S.K.: Some thoughts on combination chemotherapy of breast cancer. In Staquet, M. (Ed.): The Design of Clinical Trials in Cancer Therapy. Brussels, Editions Scientifiques Europeennes, 1972.

7. Carter, S.K.: Study design principles for the clinical evaluation of new drugs as developed by the chemotherapy program of the National Cancer Institute. In Staquet, M. (Ed.): The Design of Clinical Trials in Cancer Therapy. Brussels, Editions Scientifiques Europeennes, 1972.

8. Broder, L.E. and Rall, D.P.: Chemotherapy of brain tumors. Prog. Exp. Tumor Research 17: 373-399, 1972.

9. Burchenal, J.H. and Carter, S.K.: New cancer chemotherapeutic agents. Cancer 30:1639-1646, 1972.

10. Carter, S.K. and Broder, L.E.: Clinical studies with the nitrosoureas: BCNU, CCNU and Streptozotocin - A new class of antitumor agents. In Semonsky, M., Hejzlar, M. and Masak, S. (Eds.): Advances in Antimicrobial and Antineoplastic Chemotherapy, Volume II. Baltimore, University Park Press, 1972, pp. 789-791.

11. Carter, S.K. DiMarco, A., Ghione, M., Krakoff, I.H. and Mathé, G. (Eds.): International Symposium on Adriamycin. New York, Springer-Verlag, 1972, 251 pp.

12. Carter, S.K., Schabel, F.M., Jr., Broder, L.E. and Johnston, T.P.: 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and other nitrosoureas in cancer treatment: A review. In Klein, G. and Weinhouse, S. (Eds.): Advances in Cancer Research, vol. 16. New York, Academic Press, 1972, pp. 273-331.

13. Friedman, M.A. and Carter, S.K.: The therapy of osteogenic sarcoma: Current status and thoughts for the future. J. Surg. Oncology 4: 482-510, 1972.

14. Goldin, A., Carter, S.K. and Schein, P.: Quantitative comparison of toxicity in animals and man. In Staquet, M. (Ed.): The Design of Clinical Trials in Cancer Therapy. Brussels, Editions Scientifiques Europeennes, 1972.

15. Carter, S.K.: The choice of therapy in the treatment of malignancy. The Medical Letter 15: 9-16, 1973.

16. Carter, S.K. and Livingston, R.B.: Single agent therapy for Hodgkin's disease. Arch. Intern. Med. 131: 377-388, 1973.

17. Slavik, M., Keiser, H.R., Lovenberg, W. and Sjoerdsma, A.: Hyperaminoacidemia in rabbits treated with 6-Azauridine triacetate. Life Sciences 10: 1293-1296, 1971.

18. Slavik, M., Keiser, H.R., and Lovenberg, W.: Changes in serum and urine amino acids in patients with progressive systemic sclerosis treated with 6-azauridine triacetate. Biochem. Pharmacol. (in press).

19. Slavik, M., Danilson, D., Keiser, H.R., and Henkin, R.I.: Alterations in metabolism of copper and zinc following administration of 6-azauridine triacetate. Biochem. Pharmacol. (in press).

20. Slavik, M. and Carter, S.K.: Bronchogenic carcinoma - new drugs available for study. Cancer Chemother. Rep. (in press).

21. Slavik, M. and Carter, S.K.: Chromomycin A_3, mithramycin and olivomycin: Antitumor antibiotics of related structure. Ad. Pharmacol. Chemother. (in press).

22. Slavik, M. and Carter, S.K.: Development and clinical pharmacology of anticancer drugs in the division of cancer treatment of the National Cancer Institute. Clin. Pharmacol. Ther. (in press).

23. Blum, R.H., Livingston, R.B., and Carter, S.K.: Hexamethylmelamine - A new drug with activity in solid tumors. Europ. J. Cancer, (in press).

24. Blum, R.H. and Carter, S.K.: Pulmonary complications of cancer chemotherapy. N. Eng. J. Med. 288: , 1973.

25. Blum, R.H., Carter, S.K., and Agre, K.: A clinical review of bleomycin - a new antineoplastic agent. Cancer (in press).

PRESENTATIONS

1. Carter, S.K., The Methodology of Clinical Trials. Presented at the E.O.R.T.C. Course in Clinical Pharmacology, Institute Jules Bordet, Brussels, Belgium, May 3, 1972.

2. Carter, S.K., The Determination of the Initial Dose in Clinical Trials. Presented at the E.O.R.T.C. Course in Clinical Pharmacology, Institute Jules Bordet, Brussels, Belgium, May 5, 1972.

3. Carter, S.K., Combination Therapy. Presented at the E.O.R.T.C. Course in Clinical Pharmacology, Institute Jules Bordet, Brussels, Belgium, May 5, 1972.

4. Carter, S.K., A Review of Single Agent Chemotherapy. Presented at the Course on Current Concepts in Clinical Oncology, Jack Masur Auditorium, Bethesda, Maryland, May 18, 1972.

5. Carter, S.K., The Approach to Gastrointestinal Cancer Therapy at the National Cancer Institute. Presented at the Postgraduate Course in Gastrointestinal Cancer, National Cancer Institute, Milan, Italy, May 23, 1972.

6. Carter, S.K., New Drugs on the Horizon in Gastrointestinal Cancer. Presented at the Postgraduate Course in Gastrointestinal Cancer, National Cancer Institute, Milan, Italy, May 24, 1972.

7. Carter, S.K., Single and Combination Nonhormonal Chemotherapy in Breast Cancer. Presented at the National Conference on Cancer Chemotherapy, New York City, June 2, 1972.

8. Carter, S.K., Some Thoughts on the Methodology of Clinical Trials in Acute Leukemia. Presented at the Workshop for Standardization of Nomenclature and Methodology for Clinical Trials in Acute Leukemia, Paris, France, June 19, 1972.

9. Carter. S.K., New Drugs in the United States. Presented at the Annual Plenary Meeting of the E.O.R.T.C., Paris, France, June 23, 1972.

10. Carter, S.K., The Methodology of Clinical Trials. Presented at the Institute for Experimental and Clinical Oncology, Moscow, Russia, June 29, 1972.

11. Carter, S.K., New Drugs Available for Hematologic Malignancies. Presented at the International Society of Hematology, Sao Paulo, Brazil, July 20, 1972.

12. Carter, S.K., New Drugs and Adjuvant Therapy. Presented at the International Symposium on Eradication of the Last Neoplastic Cell, Rio de Janeiro, Brazil, July 24, 1972.

13. Carter, S.K., The Current Status of Chemotherapy. Presented at the Denver Children's Hospital, Denver, Colorado, August 22, 1972.

14. Carter, S.K., Where are We Today in Cancer Therapy? Presented at Medical Grand Rapids, Fitzsimmons General Hospital, Denver, Colorado, August 23, 1972.

15. Carter, S.K., Study Design Principles in Chemotherapy. Presented at the Brain Tumor Study Group Meeting, Baltimore, Maryland, September 15, 1972.

16. Carter, S.K., An Overview of the Status of the Nitrosoureas. Presented at the Third Joint Working Conference at the Hunt Valley Inn, Maryland, October 2, 1972.

17. Carter, S.K., An Overview of Therapy Plans in Gastrointestinal Cancer. Presented at the Third Joint Working Conference at the Hunt Valley Inn, Maryland, October 3, 1972.

18. Carter, S.K., An Overview of Clinical Experience with Adriamycin. Presented at the Third Joint Working Conference at the Hunt Valley Inn, Maryland, October 3, 1972.

19. Carter, S.K., Chemotherapy and Management of Advanced Breast Cancer Lesions. Presented at the Vanderbilt University School of Medicine, Symposium on Cancer of the Breast, Nashville, Tennessee, October 14, 1972.

20. Carter, S.K., Report on the NCI Conference on BCG. Presented

at the International Workshop for Therapy of Lung Cancer, Airlie House, Virginia, October 17, 1972.

21. Carter, S.K., Some Thoughts on Stage IV Patients with Bronchogenic Carcinoma. Presented at the International Workshop for Therapy of Lung Cancer, Airlie House, Virginia, October 18, 1972.

22. Carter, S.K., Prospectives for a Cooperative Clinical Immunochemotherapy Program in Leukemia. Presented at the Postgraduate Conference on Immunochemotherapy of Cancer held at the Mount Sinai School of Medicine, New York City, November 8, 1972.

23. Carter, S.K., Study Design Principles for the Evaluation of New Anticancer Agents. Presented at the Annual Meeting of Southwest Section, American Association for Cancer Research, Oklahoma City, November 17, 1972.

24. Carter, S.K., Experimental Design Considerations in the Phase II Clinical Trial. Presented at the Institute for Experimental and Clinical Oncology, Moscow, U.S.S.R., December 1, 1972.

25. Carter, S.K., Study Design Principles for New Anticancer Drugs. Presented at the Institute of the Problems of Oncology of the Academy of Science of the Ukrainian S.S.R., Kiev, U.S.S.R., December 6, 1972.

16. Carter, S.K., Study Design Principles for New Anticancer Drugs. Presented at the Petrov Institute, Lenigrad, U.S.S.R., December 8, 1972.

27. Carter, S.K., Drug Development Programs - Progress Report on Recent Developments in Europe, Asia, and Moscow. Presented at the American Association for the Advancement of Science Symposium of the National Cancer Program and Its International Aspects, Jack Masur Auditorium, Bethesda, Maryland, December 27, 1972.

28. Carter, S.K., Breast Cancer Chemotherapy Studies in the Division of Cancer Treatment. Presented at the First Annual Working Conference of the Breast Cancer Task Force, Williamsburg, Virginia, February 6, 1973.

29. Carter, S.K., An Overview of the Disease-Oriented Combined Modality Approach of the Division of Cancer Treatment, NCI. Presented at the Cancer Treatment Advisory Committee Meeting, Bethesda, Maryland, March 1, 1973.

30. Carter, S.K., The DCT Overall Program in Gastrointestinal Cancer. Presented at the Cancer Treatment Advisory Committee Meeting, Bethesda, Maryland, March 1, 1973.

31. Carter, S.K., The Interactions of the DCT with other NCI Treatment Programs. Presented at the Cancer Treatment Advisory Committee Meeting, Bethesda, Maryland, March 1, 1973.

32. Carter, S.K., An Overview of the Clinical Situation with Adriamycin.p Presented at the Cancer Treatment Advisory Committee Meeting, Bethesda, Maryland, March 2, 1973.

33. Carter, S.K., The Approach to Pancreatic Cancer in the Division of Cancer Treatment. Presented at the Ad Hoc Pancreas Working Group Meeting at the Holiday Inn, Bethesda, Maryland, March 6, 1973.

34. Carter, S.K., The DCT Combined Modality Approach with Emphasis on Gastrointestinal Cancer. Presented at the Eastern Cooperative Oncology Group Meeting, Miami, Florida, March 13, 1973.

35. Carter, S.K., The Clinically Oriented Approach to Experimental Studies of Combination Therapy. Presented at the Interim Screening Workshop of the Drug Evaluation Branch, Southern Research Institute, Birmingham, Alabama, March 19, 1973.

36. Carter, S.K., Adriamycin - An Exciting New Antitumor Drug and Its Integration into New Cancer Therapy Approaches. Presented at the American Cancer Society's Science Writers Seminar, Nogales, Arizona, April 4, 1973.

37. Slavik, M., Chromomycin A_3. Presented at the Phase I-II Liaison Meeting, NCI, NIH, Bldg. 31, Conference Room, March 9, 1972.

38. Slavik, M., New Drugs for Phase I-II Study, Chromomycin A_3 and Isophosphamide. Presented at the SEOG Meeting, Boston, May 2, 1972.

39. Slavik, M. and Carter, S.K., Single Agents in Prostatic Cancer. Presented at the National Prostatic Carcinoma Program, Treatment Subcommittee Meeting, Hulburt Room, Greg Bldg., Mass. General Hospital, Boston, May 6, 1972.

40. Slavik, M., Cytembena (NSC 104801). Presented at the Decision Network Meeting, May 15, 1972.

41. Slavik, M., 5-Azacytidine (NSC 102816), Isophosphamide (NSC 109724) and cis-Platinum Diamminodichloride (NSC 119875) - New Drugs for Phase II Study. Presented at the VA Lung Group Meeting, Miramar Hotel, Santa Monica, California, September 25, 1972.

42. Slavik, M., Bronchogenic Carcinoma - New Agents Available for Study. Presented at the International Workshop for Therapy of Lung Cancer, Airlie House, Virginia, October 17, 1972.

43. Slavik, M., Antineoplastic Drugs - Dermatologic Implications. Presented at the 31st Annual Meeting American Academy of Dermatology, Symposium on How Drugs Work, Bal Harbour, Miami Beach, Florida, December 5, 1972.

44. Slavik, M., 5-Azacytidine, Summary on Phase I Studies. Presented at the Western Cancer Study Group Meeting, Jack Tar Hotel, San Francisco, Cslifornia, January 12, 1973.

45. Slavik , M., Cytembena (NSC 104801), Tilorone (NSC 143969), Evaluation of Results from Clinical Studies. Presented at the Decision Network Meeting, January 15, 1973.

46. Slavik, M., Analysis of Side effects of Drugs in Man as an Implement for Experimental Therapy. Presented at the Michigan Research Foundation, Wayne State University, Detroit, January 31, 1973.

47. Slavik, M., Fluorodopan (NSC 73745), Asaley (NSC 167780), Diobenzotepa (NSC 167781), Megadoses of Vitamin A (emulsion), Hycanthone (NSC 142982) - Evaluation of Clinical Data. Presented at the Decision Network Meeting, March 5, 1973.

48. Slavik, M. and Carter, S.K., Drug Development and Clinical Pharmacology in the Division of C ncer Treatment of the National Cancer Institute. Presented at the Merck Fellow Symposium, Clinical Pharmacology Session, Rahway, New Jersey, Auditorium, Bldg. 80, March 19, 1973.

49. Blum, R.H., Bleomycin: A Review of All Clinical Trials, Response and Toxicity. Presented at the Seventh Joint Meeting of the Clinical Society and Commisioned Officers Association, USPHS, New York, New York, May 30, 1972.

50. Blum, R.H., The Pulmonary Complications of Cancer Chemotherapy, American College of Chest Physicians, Denver, Colorado, October 25, 1972.

51. Comis, R.L., Chemotherapeutic Agents of Potential Interest in Gynecologic Cancer. Presented at the Gynecology Oncology Group, New Orleans, November 17, 1972.

52. Comis, R.L., New Agents Committee Report. Presented at the Acute Leukemia Group B Meeting, Holywood, Florida, December 2, 1972.

CLINICAL TRIALS SURVEILLANCE SECTION

Clinical Trials Surveillance Section provides scientific and technical writing, editing, and related expertise, as well as overall technical information services supporting Cancer Therapy Evaluation Branch.

Through the Literature Research Section, Program Analysis Branch, literature searches are in process or have been completed on subjects of broad interest to Branch activities, such as the following:

1. Surgery, radiotherapy, and chemotherapy of pancreatic cancer.
2. Natural history of pancreatic cancer
3. Chemotherapy of prostatic carcinoma
4. Chemotherapy of Wilms' tumor
5. Vitamin C associated with cancer therapy
6. Chemotherapy of colon cancer
7. Non-hormonal drug therapy of endometrial cancer
8. Natural history, surgery, radiotherapy, and chemotherapy in stomach cancer.

Considerable effort has been devoted to the manual files on treatment protocols being used by the cooperative clinical groups, specifically to insure that current results are available for each protocol. This effort is being coupled with record maintenance and plans for improving the cycle for acquisition of data that possibly will serve as a basis for automating some portions of information storage and retrieval.

By far the major effort is in writing and editing of a broad variety of publications and presentations, and publishing the minutes and proceedings of numerous meetings such as:

1. Minutes - Gastrointestinal Tumor Study Group
2. Minutes - Testicular Tumor Study Group
3. Minutes - Osteosarcoma Study Group
4. Proceedings - Phase I-II Liaison Meeting (March, 1972)
5. Proceedings - New Drug Liaison Meeting (Feb., 1973).

It is felt that this service greatly reduces the amount of time spent in these endeavors by the CTEB clinical staff and improves the quality of the information and the speed of its dissemination.

DRUG LIAISON AND DISTRIBUTION SECTION

The Drug Liaison and Distribution Section is responsible for compliance with Food and Drug Administration regulations regarding the use of investigational anticancer drugs in man, and for the supply and distribution of both investigational and commercially available drugs to various clinical investigators throughout the United States.

In this section investigational new drug applications (INDs) are prepared and submitted to the FDA in order to initiate clinical trials in man. At the present time the NCI has 102 active INDs on file with the FDA. During the past year 260 new investigators requested drugs, which was reflected by the increased number of protocols submitted and by the number of drug requests processed each day by the section. An average of 42 drug requests is now processed daily. Each drug request varies from one drug with one dose formulation to 5 or 6 drugs which may have more than one dose formulation. Since the 102 drugs for which there is an active IND comprise about 200 dose formulations, the processing of a large number of drug requests can become a very time consuming problem. In order to facillitate the processing of drug requests and increase efficiency, steps have been taken to install a computer terminal in the section.

The supply and distribution of investigational drugs may present complications since each drug is unique in its synthesis and dose formulation. Supply and distribution may be further complicated when the drug enters Phase I clinical trial and the maximum tolerated dose in man is much greater than anticipated from the animal data. The section works very closely with members of the NCI staff who are involved in the procurement, synthesis, screening, formulation, pharmacology, and toxicology of drugs, and plans for the clinical drug requirements with the chairmen of the major cooperative study groups. This knowledge and planning enabled the section to very effectively manage a drug crisis which occurred when shipments of Adriamycin to the U.S. suddenly stopped.

The section also serves as an interface between the various clinical investigators and NCI staff regarding problems which arise concerning drug stability, purity, and dose formulation. The Head, Drug Liaison and Distribution Section, is a member of the following drug-oriented committees:

1. Operating Committee (Executive Secretary)
2. Decision Network Committee
3. Drug Evaluation Committee
4. Drug Status Committee
5. Quality Control Committee

The section also advises independent investigators in private practice about FDA guidelines on anticancer drugs.

During the past year, Drug Liaison and Distribution Section prepared and filed IND applications with the FDA for the following drugs:

1. VM-26 (NSC 122819) is a semisynthetic epipodophyllotoxin. It is a derivative of podophyllotoxin, derived from the roots of Podophyllum peltatum (Mandrake, May Apple). VM-26 is unique among podophyllum lignanes because of its antitumor activity in mice with IP- and IC-innoculated L1210 leukemia and in rats bearing the Walker carcinoma. In IP-inoculated L1210, VM-26 produces > 500% ILS when administered at 1 mg/kg every 3 hours on day 1 and day 9, or on days 1,5, and 9. An ILS of 36%-56% has been reported against the IC-innoculated L1210. It is believed that the drug exerts its cytotoxic effect by inhibition of DNA synthesis. Preclinical toxiciology studies indicate enterocolitis, lymphoid involution, and myelosuppression in rats and monkeys, but a lack of cardiovascular toxicity in dogs and monkeys.

In man, the drug has undergone clinical trial at three different dose regimens. These were (1) 30 mg/m^2/d x 5 d q 21 d, (2) 70 mg/m^2 - 130 mg/m^2/wk x 6-8 wks, and (3) 40-115 mg/m^2 twice weekly. Leukopenia was the main consistently observed dose-limiting toxicity and was reversible. Nausea and vomiting occasionally occurred at high doses but were not dose-limiting and did not result in weight loss. This drug causes hypotension if administered by rapid IV push and the schedules used employed a 30 - 60 minute infusion time.

The drug has been reported to have clinical activity in Hodgkin's and non-Hodgkin's lymphomas, astrocytoma, and possibly carcinoma of the bladder. In hematologic malignancies, it is reported to be inactive in ALL but possible activity has been reported in AML. At the present time, the drug is undergoing Phase II clinical trials in patients with intracerebral malignancy at the NCI-BCRC. The drug dosage being employed is 100 mg/m^2/week for 6-8 weeks.

2. VP-16 (NSC 141540) is structurally and chemically very similiar to VM-26. In L1210 leukemia. VP-16 shows an ILS of 297% when administered at a dose of 38 mg/kg IV twice weekly for 3 weeks. However, compared to VM-26, the drug is inactive against the IC-innoculated L1210. In mice given VP-16 IP daily x 5 days, the LD10 has been reported to be 7.7 mg/kg, and the LD50 has been reported to be 10.9 mg/kg. VP-16 is thought to exert its antineoplastic activity through the inhibition of DNA sythesis, with minimal effect on RNA and protein synthesis.

In rats treated daily for 4 weeks at a dose of 6 mg/kg IP, there was a marked effect on the hemopoietic and lymphopoietic systems. In rhesus monkeys a dose of 0.4 mg/kg IV daily for 4 weeks was well tolerated, 1.2 mg/kg induced slight anemia and leukopenia, and 3.6 mg/kg led to severe hemopoietic toxicity, but no deaths occurred.

In man the drug has been administered on a daily schedule and a biweekly schedule. The daily schedules employed were 45 mg/m^2/d x 7

and 60 mg/m^2/d x 5 q 21 days for 2-6 cycles. The biweekly schedules employed 86 mg/m^2 biweekly for 3 weeks and 300 mg/m^2 biweekly. The toxicities observed in man included reversible leukopenia and thrombocytopenia, alopecia, and nausea and vomiting. Hypotension was also observed despite slow IV infusion.

VP-16 has been reported active in lymphomas and in acute monocytic and myelomonocytic leukemia. The drug reportedly is not active in breast cancer or head and neck cancer. At present, VP-16 is undergoing Phase II clinical trial at Roswell Park Memorial Institute, Buffalo, New York, and at the NCI-VA Medical Oncology Service. The dose regimen at both institutions is 70 mg/m^2 IV twice weekly.

3. d-Tetrandrine (NSC 77037). d-Tetrandrine is a cytotoxic bis-benzylisoquinoline plant alkaloid derived from the root of Stephania tetrandra, a plant which grows in India. This drug demonstrated activity in the Walker 256 experimental system on both a daily schedule and on day 1 only. When administered IP at a dose of 128 mg/kg q d 1-9 an ILS of > 252% was obtained, and when administered at the same dose and route on day 1 only the ILS was > 287%. The daily x 9 schedule produced 40% (4/10) 40-day survivors, and the day 1 only schedule produced 50% (5/10) 40-day survivors.

When evaluated for activity against human carcinoma of the nasopharynx carried in KB cell culture, d-Tetradrine showed an ED50 of 0.091 at 0.17 mcg/ml. The LD50 for rodents is reported to be 950 mg/kg, and is somewhat greater than the apparent LD50 for dogs and monkeys, which is 50 mg/kg and 100 mg/kg respectively. The exact mechanism of the cytotoxic effect of this drug is not yet clear and is under study.

Initial preclinical toxicologic studies carried out in dogs and monkeys confirmed previous reports of the potential cardiotoxicity of this drug. Cardiovascular reactions include development of bradycardia, an initial decrease in diastolic pressure, and decreases in peripheral vascular resistance, mean arterial blood pressure, and systolic pressure. Also, EKG alterations suggest evidence of heart block. In addition, high doses appear to depress respiratory function. The dog and monkey appear to be equally sensitive to the cardiovascular effects, which can could be circumvented by slow infusion in a range from 5.0 mg/min to 12.5 mg/min. Three of six (3/6) dogs who received the drug by IV injection at 95 mg/min expired due to cardiovascular reactions, whereas only 2/28 dogs who received IV infusions at 12.5 mg/min expired with acute cardiovascular complications. Experimental antidotal procedures in several mongrel dogs indicate apparent correction of the cardiovascular reaction and heart block with the simultaneous infusion of d-Tetrandrine and norepinephrine.

Some of the more significant pathologic manifestations of d-Tetrandrine toxicity, other than cardiovascular, include signs of swelling,

edema, and ulceration at the site of injection. Alteration of liver function parameters are also observed. This latter toxicity is in general reversible and consistently occurs regardless of the dose schedule. A mild reduction in circulating lymphocyte counts has been correlated with histopathologic findings of generalized necrosis of lymphoid germinal centers. Hematopoietic toxicity includes a myelostimulatory-like effect, increases in immature neutrophil counts, and increased numbers of eosinophils in bone marrow smears. Mild anemia is evident at higher doses. The drug is formulated for parenteral use in 50 mg and 500 mg vials.

Three institutions of the Eastern Cooperative Oncology Group have initiated Phase I clinical trial with this drug using two different dose schedules, i.e., a single dose schedule and a daily x 5 schedule.

4. MER BCG (NSC 143769) is the methanol extraction residue of BCG, the Bacillus of Calmette-Guerin. One of the major problems in the production of immunity in man with whole killed tubercle bacilli is the toxicity of the vaccine. In 1955 it was demonstrated that by extraction with solvents, toxic factors could be separated from the factors which are responsible for the immunity. Extraction with methanol yields a residue that has little toxicity, if any, and can be used to immunize mice against tuberculous.

In 1959 it was reported that intact tubercle bacilli of the BCG strain retarded the progress of several non-isologous tumors in mice. This protective action was shown against both experimentally induced tumors and spontaneous tumors. The tumors which were tested included a hepatoma, osteogenic sarcoma, and a uterine sarcoma. It has also been shown that MER can increase the resistance of mice to such organisms as Klebsiella, Pseudomonas species, Salmonelli typhi, staphylcocci, and streptococci.

In the mid-1960s it was shown that MER BCG retarded or prevented the development of spontaneous mammary carcinomas in C3H mice. However, high doses of MER appeared to accelerate tumor growth. The mechanism of action of MER BCG is not yet clear, and suggestions that MER BCG might play a role in how an antigen is formed require further work. Clinical data relating to MER BCG administration is scanty, but at least four patients have been treated in Israel. These patients, diagnosed as AML, were induced with chemotherapy and then were given MER BCG at the 1 mg level and 2 mg level. Of the two patients who are still alive, one remains in complete remission 10 months after initiation of MER BCG; the other is still in complete remission 14 months after initiation of MER BCG. The toxicities reported include inflammatory lesions at the injection site, occasional regional lymphadenopathy, and occasional low to moderate grade fever. Clinical trials are currently proceeding in Israel and will soon start in this country.

5. Cyclocytidine (NSC 145668) is an anhydrous derivative of cytosine

arabinoside (Ara-C, NSC 63878) which is resistant to deamination by pyrimidine nucleoside deaminase. Ara-C is a drug of major importance in the chemotherapy of acute leukemia and it is known that the schedule of administration is of great importance in determining the efficacy of the drug. However, since Ara-C is rapidly inactivated in man by deamination, it has been administered by 120-hour IV infusions or IV injections given every 6-8 hours in order to achieve sustained therapeutic levels. Workers in the U.S. and Japan have suggested that cyclocytidine may act as a slow-release form of Ara-C since it is resistant to deamination. Available evidence to date indeed indicates that cyclocytidine hydrolyzes to Ara-C, which is then converted to phosphorylated nucleotide. Thus cyclocytidine, like Ara-C, exerts its cytotoxic mechanism of action through inhibition of DNA synthesis.

Optimal daily cyclocytidine treatment was superior to optimal daily Ara-C treatment when given IP to mice with early IP-implanted L1210. Cyclocytidine produced an ILS of 272% and 3/8 60-day survivors when administered at the OD of 400 mg/kg/d x 9, whereas Ara-C produced an ILS of 78% and no 60-day survivors when given at the OD of 80 mg/kg/d x 9. Cyclocytidine is at least as effective as Ara-C on the Q3H/24H/Q4D schedule. Also, daily treatment with cyclocytidine was more effective than daily treatment with Ara-C in advanced L1210 leukemia and against IC-implanted L1210 (145% ILS vs 95% ILS). A variant of L1210 developed for resistance to Ara-C was cross-resistant to cyclocytidine.

In Japanese pre-clinical toxicity studies, monkeys and dogs were given IV injections of cyclocytidine for 7 consecutive days. No serious side effects were reported in monkeys at a dose of 20 mg/kg/d. Vomiting occurred at twice this dose in the dog. No significant toxicity in the liver and kidney was seen in dogs and monkeys who were treated at 40 mg/kg/d, but slight increases in BUN were noted. Hematological observations in the monkey showed minimal effects at 80 mg/kg/d, but a slight decrease in the erythroid elements of the bone marrow was observed. The decrease in WBC counts recovered by day 35. However, abnormal values of BUN and serum alkaline phosphatase were observed at this dose level. At 160 mg/kg/d, 3/5 monkeys died on day 8 to day 9. All dogs (4/4), died on day 7 to day 9 when treated at half this dose. It was concluded that the cumulative toxicity of cyclocytidine in monkeys and dogs appears to be less prominent than that of Ara-C.

The drug has undergone clinical trials in Japan in patients with acute leukemia who received doses as high as 100 mg per day for 12 days or 1200 mg for 8 days. The drug appeared to be well tolerated with toxicity similar to that seen with Ara-C (myelosuppression and GI disturbances). Other toxicities included parotid pain and conjunctivitis. In two complete and two partial remissions reported, none of the four patients had prior Ara-C therapy. The Japanese felt that the hematologic toxicity of cyclocytidine on a milligram basis was considerably less than that seen with Ara-C. In this country,

Phase I clinical trials are underway at the M.D. Anderson Hospital and Tumor Institute in Houston.

PRESENTATIONS

1. Penta, J.S. and Goldsmith, M.A.: A Review of Azaserine (NSC 742) in Light of Current Problems in Phase I Study. Presented at The New Agents Committee of the Central Oncology Group, September, 1972.

2. Penta, J.S.: (a) Pre-clinical Toxicology of Cyclocytidine and Neocarzinostatin (b) Parenteral Dose Formulation Problems of Arabinosyl Adenine. Presented to the Southwest Cancer Chemotherapy Study Group, New Orleans, Louisiana, Ocotber 4-6, 1972.

3. Penta, J.S.: Comparison Between the Pharmacology and Biochemistry of Adriamycin and Daunomycin in Man. Presented at the Wester Cancer Study Group, January 12-13, 1973, San Francisco, California.

4. Penta, J.S.: Status of Supply and Formulation Problems with Methotrexate. Presented at the Chemotherapy Program Osteosarcoma Meeting, National Cancer Institute, Bethesda, Maryland, March 8, 1973.

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